



XVII IBC 2005

100 Years after the I IBC in Vienna 1905

XVII International Botanical Congress

Vienna, Austria, Europe

Austria Center Vienna

17 – 23 July 2005

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ABSTRACTS



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Nomenclature Section

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ABSTRACTS

Opening Lecture

Plant biology in the 21st century

D. H. von Wettstein;

Washington State University, Pullman, WA, United States.

Since the second half of the 20th century plant biological research is characterized by a period of strong convergence. Genetics, physiology, biochemistry, biophysics and other sub-disciplines of biology are joined in the common goal for clarification of the molecular processes behind the function of organelles, cells, organs and organisms. The whole chain from the information in the genome to properties and function is analyzed with sophisticated methods. Surprisingly, *Arabidopsis*, rice and *Homo sapiens* have only 30,000 genes. Analyses of the genomes reveal that different combinations of a large but limited number of protein domain units form the basis for the variation and development of the plants and animals as we see them. With the increasing and exciting information accruing from studies with model organisms, plant biologists now have the important and very urgent task to translate the benefits of this knowledge into better crop plants to combat hunger, destruction of the environment, and waste of non-renewable resources on our planet. Examples how this can be done will be presented.

General Lectures

GL1.1 Angiosperm evolution and classification from the perspective of DNA data: a review of 15 years of progress

M. W. Chase;

Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom.

Gene (DNA) data have been used extensively in studies of angiosperm evolution for only the past 15 years, but the impact of this approach has been immense. Previous classifications of angiosperms relying upon largely intuitive weighting of the available data from biogeography, morphology and phytochemistry could not be used to frame research in other areas of scientific study because they reached different conclusions. Analyses of DNA data and non-molecular data avoided the subjectivity of previous classifications. In 1998, the Angiosperm Phylogeny Group published a classification of the angiosperms that relied primarily on the molecular studies, and this set the stage for advances along several lines. The first involved molecular clock studies, which set the time frame for angiosperm diversification, and this has been extended into many other areas. Ideas about evolution of several groups of angiosperms have been drastically revised, none more so than Orchidaceae.

GL1.3 Allelopathy: challenges and achievements

Inderjit;

University of Delhi, New Dehli, India.

Allelopathy as the suppression of seed germination and growth of one species by another through the release of chemical compounds. Few studies are acknowledged to have demonstrated probable allelopathy in nature, most appeared limited to in vitro circumstances. Allelopathic effects are often modified by additional biotic and abiotic stress factors, uncertain climatic events or, edaphic factors all of which can influence the concentration and fate of allelopathic compounds in the environment. One of the major concerns about allelopathy research is inadequate methodology. However, there are some good examples providing a solid basis for inferring the significance of allelochemicals in explaining plant dominance in certain natural situations. Allelopathy as a science has certainly progressed the way beyond the days of Harper's criticisms. Allelopathy can be better conceptualized in terms of soil chemical ecology. Allelochemicals have been shown to play an important role in natural control of agricultural pests. While the major challenge is to establish the role of allelochemicals in natural conditions, the major achievement of allelopathy is its role in natural weed management.

GL1.5 Learning from enclosures: large-scale controlled environment facilities for experimental ecosystem and global change research

B. Osmond;

Forschungszentrum Jülich, Jülich, Germany.

A few decades of plant ecophysiological research with small, enclosed, controlled environment measurement devices have helped establish that, for all intents and purposes, Earth is an enclosed system made habitable over evolutionary time scales by the plant biosphere on land and sea. This paper makes the case for substantial expansion in the size and diversity of enclosed facilities for experiments in botanical research that will improve our ability to anticipate, and wherever possible, take advantage of, responses of the plant biosphere changing global climates. In the „Anthropocene“ it is already clear that we have left the domain from which we can expect to anticipate future responses of the Earth's ecosystems from past observations over the period of human evolution (Falkowski et al Science 2000, 290, 291-296), from contemporary small scale experiments, or from simply monitoring the uncontrolled experiment in global climate change. Some believe we are stalled on opposite sides of the Darwinian-Newtonian divide in Earth Systems science (Harte 2002, Physics Today October 29-34) as we try to deal with biocomplexity in ecology on the one hand and uncertain models in fluid dynamics on the other, and that we need experiments to bridge the divide. We must move rapidly to devise enclosed, controlled environment measurement systems at scales large enough to allow us to exercise our imaginations in experiments that bridge the Darwinian-Newtonian divide. Some evidence will be presented in support of this case from experience in the Biosphere 2 Laboratory 2001-2003. Without a large and sustained effort of this sort, we seem destined to deal with closure of a different kind from the presently inexorable, and largely irreversible, experiment in global climate change.

GL2.1 Pollen and spores: microscopic keys to understanding the earth's biodiversity

S. Blackmore;

Royal Botanic Garden Edinburgh, Edinburgh, United Kingdom.

The most distinctive feature of planet Earth is that, unlike any other in this solar system, it is rich in biodiversity. Our species, which evolved as part of the biosphere that sustains us, has the intelligence and curiosity to explore the world around us and to understand its complexity. Given the environmental challenges that lie ahead we have much to learn by exploring all aspects of biodiversity. One astonishingly informative field of investigation is palynology, the study of the pollen grains and spores of plants. These microscopic, self-contained biological units are surrounded by chemically resistant cell walls with distinctive structures and symmetry. They provide insights into such fundamental questions as how and when plants first colonised the land or how the earth's vegetation has developed through geological time and on finer time scales. They provide phylogenetic evidence important in plant systematics and model systems for understanding plant development at the cellular level. This will be a personal account of the interest and importance of these microscopic keys to understanding the Earth's biodiversity.

GL2.2 Short silencing RNA: the dark matter of genetics?

D. Baulcombe; The EMBO Lecture

John Innes Centre, Norwich, United Kingdom.

RNA silencing is a newly discovered mechanism of genetic regulation in eukaryotes. Six years ago the evidence for RNA silencing was based on mysterious experimental observations in plants, a fungus and in *C. elegans*. Today we know that this process has natural biological roles and we have an outline understanding of the mechanisms. In addition RNA silencing is part of powerful enabling technologies for functional genomics. The story of this recent progress is revealing about the way that science accommodates and adapts to unexpected results. It also illustrates the power of combined molecular, genetic and biochemical approaches to problems in biology. In my lecture I shall summarise key discoveries in the elucidation of the silencing mechanisms. I shall also describe findings about diverse silencing mechanisms affecting genetic regulation at the DNA and RNA levels and affecting processes including virus protection, genome maintenance and control of gene expression in response to environmental and developmental stimuli.

GL2.3 Spread and impact of alien plants: fear, data, and uncertainty

M. Rejmanek;

Section of Evolution and Ecology, University of California, Davis, CA, United States.

Biological invasions (spread of non-native organisms introduced by people) currently represent a very popular topic. There is even a certain hysteria about alien organisms, of which only some are conclusively harmful. Several hasty and premature generalizations have been made. However, biological invasions are very often just symptoms of other man-caused changes in our environment. Local anthropogenic enrichment of floras by alien species is a real phenomenon, but many naturalized plant species are probably benign. On the other hand, it is well known that some invasive plant taxa have a strong tendency to form monodominant stands and that some invaders can alter whole ecosystems. Nevertheless, reliable data on the harmful impacts of "exotic pest plants" are often missing. We have made a substantial progress in predictions of plant invasiveness and invasibility of ecosystems, but very little in predicting the environmental impacts of invasive plants. As scientists we should do our best to make it clear what we do know and what we do not know. Doing otherwise would only threaten our credibility.

GL2.4 Nod factor signaling during subsequent steps of root nodule formation

T. Bisseling;

University of Wageningen, Wageningen, The Netherlands.

The root nodule symbiosis of *Rhizobium* bacteria and legumes is set in motion by rhizobial Nod factors. These are lipochito-oligosaccharides that are secreted by the bacteria. These molecules induce the curling of root hairs, the start of the infection process as well as the mitotic activation of cortical cells. The latter results in the formation of nodule primordia that upon infection by the rhizobia develop into a nodule. A small set of legume genes is specifically involved in perception and transduction of Nod factors and several of these genes have recently been cloned. During this talk the role of the Nod factor signaling genes during root hair curling, and infection thread and nodule primordium formation in *Medicago* is discussed. *Medicago* forms indeterminate nodules. These nodules have a persistent meristem that adds cells to the different tissues of the nodule. The central tissue contains the cells that are infected by rhizobia. Infection threads grow towards the dividing meristem cells and after entering these cells bacteria are released from the threads. The Nod factor signaling genes are expressed in the cell layers of the nodule where infection threads grow and the bacteria are released. By reducing the expression level of Nod factor signaling genes it is shown that they are essential for the release of rhizobia from the infection threads.

GL2.5 Mechanisms of plant response to global atmospheric change.

S. P. Long¹, E. A. Ainsworth¹, C. J. Bernacchi¹, A. D. B. Leakey¹, P. B. Morgan², S. L. Naidu¹, A. Rogers³, H. J. Bohnert¹, D. R. Ort¹, R. L. Nelson¹; sponsored by *Plant, Cell and Environment*

¹University of Illinois, Urbana, IL, United States, ²USDA-ARS, Raleigh, NC, United States, ³Brookhaven National Laboratory, Upton, NY, United States.

The CO₂ and ozone (O₃) concentrations of the troposphere are rising with direct impacts on plants. O₃ currently costs crop production > 5bn Euro a year with parallel damage to natural ecosystems. In the short-term, elevated CO₂ stimulates and elevated O₃ depresses photosynthesis in highly predictable ways. Longer-term effects are less predictable, but new patterns are now emerging via meta-analysis of realistic field treatment in Free-Air Concentration Enrichment (FACE) facilities. The chain of effects from gene expression to acclimated phenotype that result from long-term growth in elevated CO₂ or O₃ will be reviewed. Significant season long increases in photosynthesis and production with CO₂ are found, with some surprising changes in plant development that were not apparent or suspected in studies with field enclosures. Season-long exposures to the moderate increases in O₃ observed in the field cause more transcripts to be down-regulated than up-regulated, cause a chronic decrease in photosynthetic capacity, largely attributable to decreased Rubisco activity, and cause slightly accelerated senescence.

GL3.1 Plant bio-inspired biomimetics: successes and challenges

G. Jeronimidis;

Centre for Biomimetics, Reading, United Kingdom.

Plants have always inspired mankind, from majestic trees to humble flowers, mostly on an esthetical level. More recently plants, plant tissues and organs at various levels of scale have provided paradigms and inspiration for many technological applications: from smart materials, to engineering structures, architecture, actuation, adaptive systems, etc. The best known example is "Velcro" but other applications emerging in various sectors. Plants are unique as effective examples of good design, i.e. biomimetic solutions. One of the main reasons for this is that materials and structures evolved by plants for their survival and strategies for adaptation are perhaps easier to translate and implement in a technological context. Without muscles, a nervous system and locomotion, plants have had to push optimisation of available resources to the limit in order to be successful. Existing achievements and future opportunities for plant-inspired biomimetics will be presented and discussed.

GL3.2 Sperm delivery in flowering plants: the control of pollen tube growth

P. K. Hepler¹, M. Bosch¹, A. Lovy-Wheeler¹, K. L. Wilsen¹, L. Cardenas^{1,2}, S. T. McKenna^{1,3}, J. G. Kunkel¹;

¹University of Massachusetts, Morrill Science Center III, Amherst, MA, United States, ²UNAM, Institute of Biotechnology, Cuernavaca, Mexico, ³Long Island University, Brooklyn, NY, United States.

Pollen tube growth, which is essential for double fertilization in flowering plants, is fast and highly polarized. Here we focus on processes that might regulate growth, including cytoskeletal dynamics, ionic gradients, and cell wall changes. Current progress has benefited from the fact that the rate of growth oscillates as do the expressions of most underlying processes. Using cross-correlation analysis to compare these oscillatory processes, we have distinguished between events that precede versus those that follow the change in growth rate. Events that anticipate growth may have a closer affinity to the central regulator than those that follow growth. We have identified three processes that anticipate growth as follows: 1) the increase in pH in the alkaline band, 2) the forward motion of endoplasmic reticulum in the apex, and 3) the increase in apical cell wall thickness. We propose that increases in pH stimulate remodeling of the actin cytoskeleton, which facilitates transport and secretion of cell wall components.

GL3.3 Comparative seed plant phylogenetics: evolutionary syndromes and phylogenetic phases

F. Ehrendorfer;

Institute of Botany, University of Vienna, Vienna, Austria.

Many DNA-supported phylogenetic trees of seed plant clades are now becoming available. They form a fascinating new basis for comparative analyses and permit the search for generalized syndromes and phases in evolution. Differences in life and growth forms, vegetative and reproductive features, types of cytogenetic changes, and eco-geographical positioning are parameters to characterize evolutionary syndromes. Slow or rapid change of taxa in time, balance between divergence and hybrid reticulation, rates of speciation versus extinction, and branching patterns of trees are linked with phylogenetic phases of clades (initial-differentiation-declining). Correlations between all these parameters and past to present environmental conditions clearly suggest extrinsic and intrinsic constraints in evolutionary processes. Examples demonstrate how comparative phylogenetics and the concepts of evolutionary syndromes and phylogenetic phases can contribute to a better understanding, typification and application of evolutionary phenomena and to their future modelling.

GL3.4 Phytohormones - research between whole plants and molecules

E. Beck;

Universität Bayreuth, Bayreuth, Germany.

Considerable progress has been made in the field of phytohormones, especially on the molecular level. For several classes of these effectors, receptors were identified and some of the signalling cascades have been at least partly elucidated. Examples will be presented in the lecture. Following the more general aspects of phytohormone cell biology, interactions between exogenous (also artificial) and endogenous

phytohormones will be discussed, especially in the context of plant ageing and senescence. Interaction of phytohormones with other signals will be briefly addressed. The lecture then switches to the significance of phytohormones at the whole plant level. Emphasis will be on cytokinins as effectors regulating plant growth in adaptation to supply of resources such as CO₂ and nutrients. Cytokinins may also be involved in the solid-substrate effect, i.e. a higher biomass production of plants growing in soil as compared to hydroponic culture.

GL3.5 Metabolomics, phenolic metabolism in *Arabidopsis*

N. G. Lewis;

Institute of Biological Chemistry, Pullman, WA, United States.

The biochemical pathway elaboration to the myriad polyphenols in the plant kingdom began with transition of plants to a land base some 400 million years or so ago. Of these, lignins represent Nature's second most abundant organic substances in vascular plants such as *Arabidopsis*. Nearly five decades ago, an unproven notion of random assembly was proposed to account for lignin biopolymer formation, even though essentially the entire basis of this working hypothesis was disproven. Yet some investigators still cling to this now disproven hypothesis (recently renamed "combinatorial" biochemistry) even today, with extravagant unproven claims that the biopolymer is composed of billions of isomeric forms! The recent ability to study biochemical processes at the single cell level, e.g. using laser micro-dissection, together with the application of metabolomics, proteomics and metabolic engineering, has permitted the study of both lignin assembly and the phenolics in developing *Arabidopsis*. The results obtained show the first evidence for *ordered lignin primary structure*, as well as assisting in the identification of the phenolics present in this species.

GL4.1 The green roof of the globe in a changing atmosphere

C. Körner;

Institute of Botany, Basel, Switzerland.

Elevated CO₂ can affect plant photosynthesis and thus the biosphere directly. Given that close to 90 % of the planet's biomass is tied up in trees, it is key to know how this large life carbon pool will respond to rising CO₂. For practical reasons the scientific exploration of this aspect of global change in large remained limited to experimental approaches using young, vigorously growing test systems. In this lecture, I will focus on growth, biomass responses and water relations of older trees. I will demonstrate the overarching significance of developmental processes and biodiversity. Elevated CO₂ is likely to enhance forest dynamics, rather than increase carbon storage. Forests carbon storage does and will depend on the size of land area they cover and on their successional stage. The slower carbon turns over and the closer forests are to a steady state carbon balance, the greater the amount of carbon they store. Thus, a global, carbon storage oriented policy will care for the protection of old growth forests, with pulp and fiber largely produced in fast rotation plantations. Their productivity can profit from elevated CO₂, provided soil nutrients permit.

GL4.2 The evolution of plant allometry: insights from mosses to trees

K. J. Niklas;

Cornell University, Ithaca, NY, United States.

All biological phenomena depend on the absolute sizes and the proportional relationships among the constituent parts of organisms. Intensive research has identified 'rules' for these scaling relations spanning 20+ orders of magnitude in body mass across aquatic and terrestrial plants and animals. A review of these rules indicates that deeply rooted, fundamental principles must dictate the expression of organic form and structure. The elucidation of these principles is therefore critically important to all biological research agenda. Although their explication remains elusive, recent developments in nitrogen and phosphorous allocation patterns at the cellular level (to proteins and rRNA, respectively) have provided recent insight, e.g., across diverse plant species, foliage N content scales as the 3/4 power of foliage P content, and P scales as the 4/3 power of total body mass. These and other N/P stoichiometric relations are used to argue that the ribosomal 'machinery' driving growth at the cellular level increases disproportionately with increasing body size. This claim

may explain some if not all of the 'canonical scaling rules' previously reported.

GL4.3 Plants, people, and phytochemicals: therapies or threats

P. A. Cox;

Institute for Ethnomedicine, National Tropical Botanical Garden, Provo, UT, United States.

Indigenous peoples use plants rich in phytochemicals. Some phytochemicals can be used for new drugs, such as prostratin from *Homalanthus nutans*, used in Samoa to treat hepatitis. The AIDS ReSearch Alliance will return 20% of its profits from prostratin to Samoa. The University of California, Berkeley will share equally with Samoa any revenues from cloning the prostratin gene sequence. Phytochemicals, however, sometimes present undetected threats. Chronic neurological disease, such as konzo (from *Manihot esculenta*), lathyrism (from *Lathyrus sativus*), and ALS/PDC (linked to *Cycas micronesica*) are caused by toxins in crop plants. ALS/PDC symptoms are similar to Amyotrophic Lateral Sclerosis (ALS), Parkinson's Disease (PD) and Alzheimer's Disease (AD). Cyanobacterial symbionts in *Cycas* roots produce BMAA, a potent neurotoxin, which is then biomagnified within the ecosystem. Whether phytochemicals represent therapies or threats to indigenous peoples, ethnobotanical studies of wellness and disease among the indigenous peoples can lead to significant pharmaceutical advances.

GL4.4 The role of correlated traits in plant-insect interactions

L. S. Adler;

University of Massachusetts, Plant, Soil, & Insect Sciences, Amherst, MA, United States.

Plant-insect interactions are thought to have played a key role in the diversification of angiosperms, due in part to selection by herbivores and pollinators on defensive and attractive phenotypes. Interactions with antagonists and mutualists have historically been studied independently. However, pleiotropy or genetic correlations between traits may constrain the ability of plants to evolve optimal solutions to selection pressures from these different interactions. For example, several studies have linked the expression of floral color traits with production of defense compounds and resistance to herbivory in leaves. In my research we have found tight correlations between alkaloid levels in leaves and nectar within and between species of *Nicotiana*, suggesting that defense traits may evolve in response to selection by pollinators as well as herbivores or pathogens. Other research shows that integration across tissues such as roots and shoots may constrain or enhance responses to different herbivores. Thus, the evolution of defense phenotypes may be best understood in light of the multi-species interactions that plants experience in nature.

GL4.5 The sweet side of economic botany: Biotechnology and molecular biology of cocoa: *Theobroma cacao*

R. Lieberei;

Biocenter Klein Flottbek, Hamburg, Germany.

Cocoa is one of the economically most important plant resources. It started its way around the world from the Amazon basin. As many tropical plant resources it was distributed to the former colonies around the tropical belt of the globe, and rising world wide consumption was answered by enlargement of plantation areas. First problems in production were caused by fungal diseases in the beginning of the 20th century, followed by virus and pests. Installation of gene banks, breeding and selection for resistance allowed for more efficient production, but it was only in the late sixties that a rational research on flavour and aroma quality started. Nowadays we are facing wide international molecular activities on gene pools, plant biotechnological studies on the right and efficient post harvest treatments, esp. fermentation processes, to improve and to maintain a stable quality of raw cocoa. New production areas develop in South East Asia, concomitant with their growing markets. The paper focuses on the biotechnological insights into quality determining post harvest processes and provides an overview over the molecular biological activities on the cocoa plant.

GL5.1 Transgenic food plants for the Third World

J. A. Thomson;

University of Cape Town, Cape Town, South Africa.

In Africa 70% of the population depends on agriculture as the sole source of income. However, Africa's crop production is the lowest in the world. Genetically modified crops are some of the tools that could improve efficiency, quality and productivity. African experts have prioritised biotechnology-derived crops as follows: insect resistant (Bt) African maize varieties, resistance to African plant viruses, resistance to parasitic weeds, decreased mycotoxin levels in maize and drought tolerant crops. Colleagues and I have developed Maize streak virus resistant plants using a severely mutated form of the replication associated protein. These are being bred into commercial varieties of maize. A consortium of African and American scientists are developing cassava resistant to the African cassava mosaic virus. A major parasitic weed is *Striga*, or witchweed. Field trials have been carried out on herbicide resistant maize with very promising results. South Africa recently began planting Bt white maize for human consumption and we are finding a decrease in the levels of post-harvest fungal infection with a concomitant decrease in mycotoxin content. Bt cotton, although not a food crop, is providing a much-needed increase in revenue to small scale farmers. The source of our genes for the development of drought tolerant maize is a South African indigenous "resurrection plant" which can lose 95% water content and, upon watering, "resurrect" in three days. Our first transgenic plants are showing resistance to water loss, heat and salt.

GL5.2 The fossil record of angiosperm evolution

D. L. Dilcher;
Florida Museum of Natural History, University of Florida, Gainesville, FL, United States.

Angiosperm flower origins are best determined from early angiosperm fossil flowers and fruits. Molecular systematics provides a framework for extant angiosperm relationships; the fossil record is used to document the age of major basal clades. Molecular clocks combined with fossil data provide minimum ages for major lineages. The basal angiosperms based on molecular data needs to be reconciled with the morphologically based fossil record. The overwhelming tendency is to find roots of modern major clades and families based upon limited and poorly defined characters of fossils. This results in overlooking much angiosperm evolution. Angiosperm paleobotany should be focused on "character based" data rather than finding the "first" or "oldest" record of extant taxa or modern families. Early angiosperm major extinct taxa are often not recognized or incorporated into angiosperm phylogeny. Ancient extinct angiosperm diversity, past abundance and distribution dominate angiosperm history during their first 70 to 90 million years. A better understanding of these extinct taxa and lineages must become incorporated into the record of angiosperm evolution.

GL5.3 Invasive plant species: a global problem or a question of perspective?

R. J. Hobbs;
Murdoch University, Murdoch, Australia.

It is generally argued that invasive plant species often have important environmental and economic impacts through their impacts in natural and managed ecosystems, and hence it is assumed that invasive species should be eliminated or controlled wherever possible. However, it has also recently been argued that human movement of species is simply part of the natural process of species migration, that invasive species actually increase diversity, and that the evidence for adverse effects of invasive species has been oversold. Certainly, there are so many non-native species in many parts of the world that control or elimination of them all is unlikely. So should we worry about invasive species, or simply take them as a fait accompli and part of the natural order? In this talk I discuss the evidence for important ecosystem and biodiversity impacts of invasive plant species, critically examine the proposition that these impacts have been oversold. In the light of this discussion, I then review common policy and management approaches to the problem.

GL5.4 Approaching a global view on plant signal transduction

H. Hirt;
University of Vienna, Vienna, Austria.

As sessile organisms, plants must continuously monitor for changes in their environment. Information transfer and processing, commonly called signal transduction, leads to modification of gene

expression and physiological adaptation. Passing information from one factor to the next most commonly occurs by phosphorylation through protein kinases. Almost 5 % of all genes code for different types of protein kinases, accounting for a total of more than 1000 protein kinases in the genetic model plant *Arabidopsis thaliana*. We are interested to study signal transduction in response to environmental stress and have recently identified signalling pathways for various biotic and abiotic stresses. Since most signalling pathways are interlinked with each other, signalling along one pathway often affects other pathways as well. To obtain a more global view on cellular signalling processes, we have established novel methods to detect the state of tens of signal pathways under any one condition and time. The uses and applications of these new tools will be discussed in the context of global environmental change in this century.

GL5.5 Are modern evolutionary theories and methods coherent?

M. Pigliucci;
Dept. of Ecology & Evolution, SUNY, Stony Brook, NY, United States.

Natural selection is often conceived of as a "force" that acts on populations through the differences in fitness between individuals; recent work by various authors points towards difficulties with the force metaphor and the attendant concept of fitness. Far from being a trivial semantic quibble, the way in which one conceives of natural selection and fitness strongly influences what one will take to be evidence for natural selection having occurred, and how one thinks of the difference between drift and selection. These issues also have implications for our ability to measure selection in natural populations, for the level of selection debate, for our ability to distinguish selection from drift, for our conception of constraints on evolutionary change, and for the way that the metaphor of adaptive landscapes is used.

GL6.2 Communicating plant cell biology by CD-ROM: information for students and a resource for teachers

B. E. S. Gunning;
Plant Cell Biology Group, Australian National University, Canberra, Australia.

A versatile user interface has been developed for presenting comprehensive pictorial information on plant cell biology in CD format, for use by students and teachers at all levels. Details are available at www.plantcellbiologyonCD.com. Light and electron micrographs of many types are included, plus anaglyph stereos, confocal animations, static and animated diagrams. Live cell phenomena which few biology students can experience directly are highlighted by inclusion of many time-lapse movies. Topics can be studied systematically (progress monitoring is provided) or by browsing via comprehensive navigation facilities. The CD set currently contains nearly 1000 "pages" (screens), many with multiple images. Concise, referenced text essays introduce many topics. All images have labels and captions. To facilitate use by teachers in tutorials and other presentations a built-in recorder allows sequences of screens to be pre-selected for subsequent display with single mouse-clicks, as in a slide-show. Sequences are preserved as text files, ready to be opened for re-use. The program can be used on individual PCs or on servers for classroom local area networks.

GL6.3 African ethnobotany: prospects and constraints

R. Bukenya-Ziraba;
Department of Botany, Makerere University, Kampala, Uganda.

Ethnobotany as a practice, in Europe, goes back to the 1st Century, A.D. It started as a descriptive discipline, but today it employs highly quantitative methods, although qualitative methods are still used. In the tropics the use of plants by man also dates to time immemorial, but as an organised discipline, serious ethnobotanical studies are recent. Although the indigenous people in the tropics are endowed with rich ethnobotanical knowledge and resources, they have mainly acted as informants for scientists from the developed countries, who have for a long time dominated ethnobotanical studies. People and Plants Initiative, which started in 1992 as a joint collaborative effort of WWF, UNESCO and Kew, was influential in the growth of ethnobotany particularly in Eastern and Southern Africa. Today Ethnobotany addresses among other things: edible wild plants; medicinal plants; fuel wood and charcoal; crafts and plants in domestic use; hidden economy and management of plant resources. Recent studies in ethnobotany

especially in Uganda will be outlined and constraints related to the study will be briefly discussed.

GL6.4 Plant phylogeography in the Alps

P. Taberlet;

Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Grenoble Cedex 09, France.

The first part of the talk will be dedicated to a general presentation of the concept of phylogeography, and to the molecular tools used in plant phylogeography. The second part will concern a review of the current results about plant phylogeography in the Alps, with a special emphasis about the relationship between putative glacial refugia and current genetic polymorphism. The main species concerned are *Ranunculus glacialis*, *Androsace sp.*, *Erithrichium nanum*, and *Phyteuma globulariifolium*. Finally, the first results of the ongoing European project IntraBioDiv will be presented. The goal of this project is (i) to assess the intraspecific genetic polymorphism of 25-30 plant species over the Alps, (ii) to precisely map the geographic distribution of about 1500 plant taxa, and (iii) to compare the two datasets in order to find possible relationships among inter- and intraspecific plant diversity.

GL6.5 Genomics and phylogeny

W. Martin;

University of Düsseldorf, Düsseldorf, Germany.

The evolution of the plant kingdom starts with the endosymbiotic origin of plastids from a cyanobacterial ancestor, an event that occurred more than 1.2 billion years ago. In the wake of that fateful symbiotic association, several thousand genes were transferred to plant nuclear chromosomes, but between ~60 and ~200 protein coding genes have remained in the plastid of various algal and land plant lineages. Because of their high gene density and conserved gene repertoires, plastid genomes are a convenient and useful tool for investigating plant phylogeny. In particular, they hold the promise of getting the backbone of plant phylogeny fully resolved for the most important evolutionary splits. Much larger numbers of site patterns are observable in plastid genome sequences as opposed to sequences from one or a few genes. But we still need to be concerned whether the models of sequence evolution currently in widespread use approximate the historical process of sequence difference accumulation in protein-coding regions sufficiently well as to give us reliable answers about plant history.

Closing Lecture

Think big in conservation - the Eurasian perspective

M. Succow;

Botanisches Institut und Botanischer Garten der Ernst-Moritz-Arndt Universität Greifswald, Greifswald, Germany.

I would like to dedicate this lecture to the protection of our Earth's vegetation, the forests and mires, the rivers and lakes, the steppes and deserts, the mountains and oceans. The conservation of the ecological benefits these ecosystems provide is essential to the future of humanity. The current degree of destruction of the natural vegetation cover forces us to protect those natural ecosystems that are hardly affected by human activities. On the other hand, also the preservation of the ecological benefits of anthropogenic ecosystems should receive highest priority. In order to achieve these goals, the monetarisation of ecological benefits will be unavoidable; in particular following benefits of the Earth's vegetation should be considered: CO₂ storage; groundwater recharge; evapotranspiration (cooling); binding and disposal of pollutants; surface stability; preservation of genetic resources. This conservation and management is supported by consolidated international (IUCN) protection categories: world natural heritage sites, national parks, biosphere reserves. Specific examples for the implementation of these programmes in Eurasia will be given, illustrating the conservation and wise use of tundra and taiga, deciduous forests, steppes and deserts, tугai gallery forests, and alpine vegetation.

Symposium Presentations

1.1.1. Changes in sphingolipid metabolism caused by disruption of *Arabidopsis thaliana* LAG1 homologues are associated with programmed cell death

J. Hille, T. Gechev, J. E. Markham, L. Bernier, M. Ferwerda; Research School GBB, Haren, The Netherlands.

Sphingolipids are important regulators of cell fate and function in animals, fungi and plants. The longevity assurance gene 1 (*LAG1*) family has been identified as essential for ceramide synthase activity and sphingolipid metabolism. T-DNA insertional mutants were isolated for all three members of the *Arabidopsis thaliana* *LAG1* gene family. Disruption of *LAG1* homologue 2 conferred sensitivity to the ceramide synthase inhibitor AAL-toxin. Challenge with AAL-toxin resulted in accumulation of free long chain bases, inhibition of inositolphosphoceramide biosynthesis and ultimately Programmed Cell Death (PCD). To obtain more insights into the nature of AAL-toxin-induced cell death and to identify genes of potential importance for PCD, we carried out transcription profiling of AAL-toxin-induced cell death in this knock out. In addition, we isolated mutants from this knock out that are more tolerant to AAL-toxin. Interestingly, some of these mutants were also more tolerant to H₂O₂-induced PCD.

1.1.2. The genetic basis of singlet oxygen-induced cell death in *Arabidopsis thaliana*

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Plants under oxidative stress suffer from damages that have been interpreted as unavoidable consequences of injuries inflicted upon plants by toxic levels of reactive oxygen species (ROS). However, this paradigm needs to be modified. Upon generation of singlet oxygen in plastids of the *flu* mutant of *Arabidopsis*, plants stop growing and die. Several suppressor mutations were identified that abrogate these stress reactions. One of the mutated genes, dubbed *Executer1*, has been characterized in greater details. The *executer1/flu* double mutant behaves like wild type despite its continuous production of singlet oxygen. Reintroduction of a wild-type copy of the *Executer1* gene into the *executer1/flu* mutant line restores the cell death phenotype of the *flu* parental line. Thus, these stress reactions formerly attributed to physico-chemical damage during oxidative stress are the result of an actively pursued genetic program that requires the *Executer1* protein.

1.1.3. Plant cell death regulators and stress tolerance

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Cellular calcium level plays an important role in death pathways triggered by apoptotic stimuli. Apoptotic death is inhibited by the decreased calcium concentration at ER lumen by over-expression of plasma membrane-localized Ca²⁺-ATPase while increase of calcium uptake into ER by over-expression of SERCA pump caused cells higher sensitive toward apoptotic stimuli. Anti-apoptotic Bax inhibitor-1 (BI-1), ER membrane protein exists in a wide range of higher organisms. Here, we found that *Arabidopsis* BI-1 interacted with calmodulin (CaM) protein via unknown protein. Yeast mutant lacking Ca²⁺-ATPase failed to survive by Bax even when AtBI-1 was expressed. Thus, intactness of this Ca²⁺-ATPase may be essential for the cell-death suppression by AtBI-1 in yeast. Addition of CPA; SERCA pump inhibitor, altered calcium response to H₂O₂ treated tobacco cells over-expressing AtBI-1, suggesting the functional relationship between AtBI-1 and SERCA pump in plant cells. On the basis of this finding the role of cell death regulators in relation to biotic and abiotic stress resistance will be discussed.

1.1.4. The ricinosome: an organelle for programmed cell death (PCD) in plants

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Ricinosomes are found in senescing nucellus and inner integument during castor bean seed development and in dissolving endosperm after mobilization of oil and protein stores during germination. Cells with these organelles undergo nuclear DNA fragmentation a landmark of PCD. A 45kDa pro-cysteine-

endopeptidase with a C-terminal KDEL ER retention signal (CysEP) in the ricinosomes is processed and released. Protein cleavage sites for the CysEP have been determined with fluorogenic peptide substrates and digests of bovine γ -casein. Based on the highest second-order kinetic constants k_{cat}/K_m for amino acids at the P1 and P2 position the covalent binding inhibitor H-D-Val-Leu-Lys-chloromethylketone was chosen as substrate analogue for replacement in the catalytic site. The CysEP with the inhibitor in the active site was crystallized and its structure solved by molecular replacement at 2Å resolution. The more open catalytic pocket of the Ricinus CysEP correlates with the extended varieties of substrate amino acid residues accommodated by this enzyme at the P1 and P1' position, as would be expected for an enzyme attacking a greater variety of proteins during PCD.

1.1.5. Interaction of light with living matter: a paradigm for cellular study

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When *Chlamydomonas* cells were exposed to 1-100 J/m² of UV-C, a cell death-like process occurred. Dying cells showed hallmarks of apoptosis; like cell shrinkage, phosphatidylserine flipping, and DNA fragmentation. The chromatin degrades in the nucleus, 'spills out' in the cytoplasm and is 'thrown out' to the periphery of the cell. We propose that *Chlamydomonas* harbors biochemical machinery for an apoptotic-like pathway to operate in response to UV-C. *Chlamydomonas* cells were trapped in an optical trap and such cells may be used as controllable micron-sized motors, to generate torques of up to 12,000 pN nm. Nickel-poisoned, *paralyzed flagella* and *deflagellated* mutant cells do not exhibit rotation, while *phototactic*, *eyespot* and *cytokinetic* mutants showed rotational speeds same as that of wild type. The origin of this rotation is entirely flagellar motor system driven. The force associated with a rotating cell (~10 pN) indicates that either the activity of 5% dynein molecules per beat cycle is sufficient to generate flagellar motion, or that a 'gear system' exists in the flagella.

1.1.6. Flooding-induced programmed cell death in pea (*Pisum sativum*) primary root apical meristems occurs mainly in the elongation zone

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In previous studies, sudden flooding of pea roots 5 d old at 25 °C frequently resulted in cessation of primary growth of pea taproots and often the curling of the root tips. TEM revealed morphological abnormalities in cell nuclei, and DNA extracted from flooded root tips ladderized on sizing gels. TUNEL in situ analysis showed the presence of hydrolysed DNA in the nuclei of affected cells. For the present study, under sterile conditions pea seeds were placed between paper and glass in 1 l tall beakers lined with filter paper that were then filled with vermiculite moistened with 375 ml of water. After 5 d the root systems were flooded. Root tips were collected after 3, 6, and 12 h. Some were prepared for light microscopy, including TUNEL, and some for confocal microscopy. Cells in the pericycle and endodermis opposite protoxylem poles in the elongation zone were the first to be affected by flooding. These cells showed the same symptoms of programmed cell death as previously reported.

1.2.1. Global patterns of gene expression in *Synechocystis* sp. PCC 6803 in response to inorganic carbon limitation and the definition of a key regulon

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Synechocystis possesses multiple inorganic carbon (Ci) uptake systems regulated according to Ci availability. Regulation of these systems and their integration with other cell functions remain to be clarified. Changes in global gene expression in response to Ci downshift and inactivation of transcriptional regulator, *ccmR* (sl1594), were observed. Ci-limitation elicited upregulation of both inducible CO₂ and HCO₃⁻ uptake systems. An alternate Type-1 NDH complex with products homologous to antiporter proteins was also upregulated. This leads us to propose that these genes encode in sodium efflux system, driven by redox energy and used to drive the Na⁺ dependent bicarbonate transporter, *SbtA*.

Inactivation of *ccmR* resulted in constitutive upregulation of *sbtA/sbtB*, *ndhF3/ndhD3/cupA/sl1735* and *slr2006-13* including *ndhD5* and *ndhD6*, indicating a role of the regulatory gene in both CO₂ and HCO₃⁻ and suggesting a regulon for an integrated system of proteins operating to concentrate Ci using redox energy transduced by Type I dehydrogenases directly as with the CUP subsystem or indirectly via a sodium gradient generated by the *NdhD5/NdhD6* subsystem.

1.2.2. A CO₂-signal transduction pathway, which regulates the carbon-concentrating mechanism by sensing CO₂ availability and cross talk with light signaling in *Chlamydomonas reinhardtii*

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Chlamydomonas modulates photosynthetic characteristics to acclimate to CO₂-limiting stress by sensing CO₂ availability and light by inducing a set of genes for carbon-concentrating mechanism. We have analyzed regulation of a gene *Cah1* for a carbonic anhydrase to elucidate the CO₂-signal transduction pathways. By using *Ars*-reporter fused with *Cah1* promoter, cis-acting elements and DNA-binding proteins involved in CO₂-regulation were elucidated (Kučho 2003). Regulatory mutants showing abnormal responses to the changes in CO₂ levels, were isolated (Yoshioka 2004). Genome wide analysis of gene expression of regulatory mutants revealed that CO₂-responsive genes were grouped into several categories (Miura 2004). Relationship between CO₂-dependent regulation and light-dependent systems will be also discussed based on the analyses of mutants. (Kučho Plant Physiol. 133:783-(2003), Fukuzawa, PNAS 98:5347-(2001), Yoshioka Plant Cell 16:1466-(2004), Miura Plant Physiol.135:1595(2004)

1.2.3. A carboxysome-based inorganic carbon concentration mechanism (CCM) in the glaucocystophyte alga, *Cyanophora paradoxa*?

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The glaucocystophyte alga, *Cyanophora paradoxa* contains plastids (cyanelles) which are surrounded by a peptidoglycan wall, a cyanobacterial heritage. It is uncertain, if *C. paradoxa* possesses an inorganic carbon concentration mechanism (CCM). The CCM is triggered by growth at ambient (0.04%) CO₂ with concomitant gene induction/repression and accumulation of Rubisco in microcompartments: carboxysomes in prokaryotes and pyrenoids in algae. We postulate that the cyanelles of *C. paradoxa* did also retain another cyanobacterial feature: the unique case of a eukaryotic carboxysome. An isolation procedure for carboxysomes was developed allowing the mass spectrometric identification of proteins other than Rubisco. Rubisco activase was imported into isolated cyanelles and was shown to integrate into carboxysomes. Two cDNA libraries of cells grown under ambient and high CO₂ were established for sequencing and annotation of more than 5000 ESTs. 2300 unique ESTs were chosen for microarray printing. Hybridizations with RNA from cells grown at high and low CO₂ were performed. Around 140 genes were found to be differentially expressed under high and ambient CO₂.

1.2.4. Transcriptome analysis of sugar signal transduction in *Arabidopsis thaliana*

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Transcriptome analyses have revealed global expression changes occurring over space and time when plants are treated with metabolites such as glucose or inorganic nitrogen. In our study of young *Arabidopsis* seedlings, which consist primarily of shoot tissues, glucose was generally found to be a more potent signal in affecting gene expression than inorganic nitrogen. Surprisingly, other than genes associated with nitrate assimilation, glucose also had a greater effect in affecting the expression of nitrogen metabolic genes than nitrogen itself. This indicates that sugar may have a significant role in maintaining nitrogen homeostasis via transcriptional control. Furthermore, sugar is capable of modulating the nitrogen response and vice versa- cluster analyses of the transcriptome data showed significant interaction between

glucose and nitrogen in regulating gene expression. The important role that glucose might have on nitrogen metabolism was further supported by the results of co-expression analyses, i.e., an over-represented proportion of nitrogen metabolic genes were co-expressed with the glucose-responsive regulatory genes.

1.2.5. Sugar and hormone signaling in *Arabidopsis*

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Sugars display hormone-like activity and regulate a wide variety of developmental processes in plants. The pleiotropic effects of sugars appear to be generated through regulation of gene expression, at least in part. Recent molecular genetic studies with *Arabidopsis* plants disclosed a crosstalk between sugar and hormone signal transduction pathways. For example, ethylene is antagonistic to glucose. The *ctr1* (constitutive triple response 1) and *ein3* (ethylene insensitive 3) mutants showed hyposensitivity and hypersensitivity to glucose, respectively. Our analyses of the crosstalk revealed that glucose promoted degradation of EIN3, a key transcription factor for ethylene signaling, while ethylene repressed the degradation of EIN3. Two F-box proteins involved in degradation of EIN3 were nuclear proteins, and hexokinase mediating the glucose signal was also detected in nuclear fraction. These suggested that the antagonistic effects of sugar and ethylene were generated through the opposite regulation of the degradation of EIN3 in nuclei. I will discuss the possibility that sugar signals might influence degradation of various transcription factors in nuclei, generating the pleiotropic effects.

1.2.6. Regulation of carbon-concentrating mechanism in response to changes in the ambient [CO₂] in marine diatom

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Marine diatom is the successful group of algae in the ocean and share about 25% of the global CO₂ fixation. It was shown previously that high affinity photosynthesis in the marine diatom *Phaeodactylum tricornutum* was induced in response to decrease in [CO₂] in the medium. This suggested that *P. tricornutum* cells might sense [CO₂] at the ocean surface directly or indirectly. A chloroplastic form of γ -carbonic anhydrase in *P. tricornutum* (PtCA1) is repressed in high CO₂ but induced in air. The 1.3 kbp 5'-upstream sequence of the gene encodes PtCA1 (*ptca1*) was isolated by inverse PCR. A series of 5'-truncated fragments of the 1.3 kbp upstream sequence was ligated with GUS reporter gene, *uidA*, and introduced into *P. tricornutum* with microprojectile bombardment. GUS reporter assays of these transformants showed that the critical CO₂-responsive region of *ptca1* promoter reside between -70 and -30 bp relative to the transcription-start site of *ptca1*. This region contained a cAMP responsive element (CRE) and a relating coactivator-binding region. The acclimation mechanisms and a possible concern of cAMP signaling in CO₂-sensing system in marine diatoms will be discussed.

1.2.7. Can QTL and microarrays help us to elucidate the genetic basis of plant adaptation to elevated CO₂

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Rising atmospheric CO₂ will have an impact on tree growth, development, productivity and survival, but despite this, very few studies have been undertaken to elucidate the genetic basis of response to elevated CO₂. Here we present data to show that poplar is a useful model for studying short- and long-term effects of global climate change on gene expression and the development of adaptive traits and that the latest technologies in genomics and molecular quantitative genetics may be applied to address this problem. In rising CO₂ we have identified important changes in adaptive traits and elucidated several QTL that are now being followed up in more detail. We have determined patterns of gene expression in elevated CO₂ using microarrays, in a long-term experiment lasting six years, providing a highly novel data-set. Important changes in gene expression include those determining transcription, development, cellular biogenesis and photosynthesis have been identified, that are consistent between years 3 and 6 of the experiment. Contrasting patterns of gene expression for leaves that are either sinks or sources of CO₂ have also been identified.

1.3.1. Regulation of the actin cytoskeleton

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One of the most unique features of plant cells is that they don't move so the development of tissues and organs is dependent on the balance of cell division, cell expansion and the positioning of the new crosswalls in cytokinesis. The plant cytoskeleton governs plant cell morphogenesis and it is composed of microtubules and actin filaments. Both cytoskeletal networks are stimulus responsive being effected by internal stimuli, by biotic and abiotic stresses in signal transduction pathways still to be fully assembled. The completion of the *Arabidopsis* genome sequence has allowed for a comparative identification of many cytoskeletal proteins and components of signal transduction cascades. These pathways have components common to both plant and animal/fungal pathways but they are not identical presumably because signals in plants are quite unique. This presentation will give an overview of the key stimulus responsive molecules of the actin cytoskeleton.

1.3.2. Actin capping protein is regulated by the stress-inducible phospholipid, phosphatidic acid

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Changes in cellular architecture often require actin-binding proteins as stimulus-response modulators of the cytoskeleton. In plants, phosphatidic acid (PA) is gaining widespread acceptance as a major signaling lipid that mediates responses to stress. To investigate the regulation of actin cytoskeleton, we analyzed the properties of a heterodimeric capping protein from *Arabidopsis*. AtCP binds with high affinity to PA, with a Kd value of 17 μ M and stoichiometry of ~1:2. It also binds well to PtdIns(4,5)P₂, but not to several other phosphoinositide lipids. The interaction with PA micelles inhibited the activity of AtCP. In the presence of PA, AtCP is unable to cap the barbed or rapidly growing and shrinking end of actin filaments and fails to nucleate filament formation. Pre-capped filament barbed ends can also be uncapped by PA, allowing rapid filament assembly from an actin monomer pool that is buffered with profilin. The findings support a model in which the inhibition of capping protein activity in plant cells by elevated PA results in the stimulation of actin polymerization from a large pool of profilin-actin.

1.3.3. Control of actin dynamics by plant formins

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Formins are actin-organising proteins involved in cytokinesis and polarity determination. In plant *Arabidopsis*, there are more than 20 formin homologues with unknown roles. We characterize biochemical properties of the *Arabidopsis* formins AtFH1 and AtFH5. Despite low similarity to yeast and mammalian formins, AtFH1 and AtFH5 proteins interact with barbed end of actin filament and nucleates actin filament polymerization in vitro. However the effect of AtFH1 and AtFH5 on actin dynamics in vitro is quantitatively different. In vivo, AtFH5-GFP fusion protein localizes to the cell plate, a plant-specific membranous component assembled at the plane of cell division. Consistent with these data, an *atfh5* loss-of-function mutation causes a cytokinesis defect in the seed endosperm. Although cytokinesis in animals, yeast and plants occurs through morphologically distinct mechanisms, our study finds that formin recruitment to sites of actin assembly is a common feature of cell division across eukaryotes.

1.3.4. Actin-annexin relationships in stress signaling

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Plant response to stresses involves rapid cytoskeleton rearrangement that form structural basis for defense response. Thus proteins that can influence F-actin remodeling in response to stress are intensively studied. In animal cells annexins, a family of highly conserved calcium binding proteins was recently suggested to play a role in actin filaments anchoring near the cellular membranes. We decided the check interactions between actin remodeling and annexins in signal transmission during plant response to stress. In preliminary experiments we found that

AnnAt1 mRNA level was induced in *Arabidopsis* plants after different biotic and abiotic stimuli. It suggests that annexins can function upstream of branching point in diverse signaling pathways. We also found that *AnnAt1* is able to bind actin *in vitro*, and this binding could be probably influenced by GTP. Actin cytoskeleton rearrangement in plants deficient in *AnnAt1* production will be studied *in vivo* with confocal microscopy. Additionally, we will perform crossing between plants with GFP-tagged actin cytoskeleton (GFP-FABD) with plant (*AnnAt1*) do not producing annexin 1.

1.3.5. Myosins of the class XI in *Arabidopsis*

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Myosins are eukaryotic molecular motors moving along actin filaments. *Arabidopsis thaliana* genome contains 17 sequences that could code myosin proteins. Only a small set of myosin classes - VIII with 4 and XI with 13 putative genes - is present in the genome. The specific functions of *Arabidopsis* class XI myosins are unclear although knockout of a single representative, *mya2* has been described (Holweg and Nick, 2004). Loss-of-function mutation in *mya2* had pleiotropic effects in plant development. *Arabidopsis* T-DNA insertional mutants (from ABRC) of myosin genes were used to study the functions of class XI myosin genes. Mutants with homozygote T-DNA insertion in 11 different class XI myosin genes have been identified. Three different T-DNA insertion mutants in a single myosin gene show similar but quantitatively different phenotypes of homozygote plants: impaired root hair cells growth. Morphometric analysis of four-day-old mutant seedlings showed that average length of root hairs was 77, 50, and 41% of wild type root hairs, suggesting involvement of this class XI myosin in cell elongation and tip growth.

1.3.6. Interaction of chloroplasts with actin filaments and plasma membrane in spinach mesophyll cells *in vivo* and *in vitro*

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In mesophyll cells of spinach (*Spinacia oleracea* L.), chloroplasts change their intracellular positions in response to illumination. Cytochalasin and BDM inhibited the movement of chloroplasts in a reversible manner. Under low-intensity white light, numerous short, thick bundles of actin filaments were observed in the vicinity of immobile chloroplasts by staining with fluorescent phalloidin. In contrast, one or two long, straight bundles of actin filaments appeared in a cell exposed to high-intensity blue light. Interaction of chloroplasts with endogenous actin filaments and the plasma membrane was examined by preparing membrane ghosts from the mesophyll protoplasts, which had been kept under various conditions. Furthermore, we isolated intact chloroplasts, which retained their outer envelopes, and revealed their ability to bind to exogenously added skeletal muscle F-actin by co-sedimentation assays and immunoblotting. After disruption of the outer envelope of isolated chloroplasts by protease treatment, actin was no longer co-precipitated with the chloroplasts.

1.3.7. A high throughput screen for new plant cytoskeletal proteins in mammalian cells

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Based on the high conservation of the basic cytoskeletal building blocks, actin and tubulin, between the animal and plant kingdoms we have developed a novel method of screening an *Arabidopsis* cDNA library fused to YFP in rat fibroblasts. Using a newly developed microscope system with computer controlled stage for multi-well plate scanning and fast laser auto-focusing for high magnification imaging we are able to screen millions of fibroblast cells expressing thousands of YFP fused *Arabidopsis* cDNAs. This special method overcomes a significant obstacle in the plant cell biology field, which is the high throughput automatic screening of thick plant cells, by using flat adherent fibroblasts cells as a host system. Using the above system, we isolated a couple of plant genes which show strong actin binding activity in fibroblasts. The

full length cDNAs of selected genes were isolated and their ability to bind actin in fibroblasts was demonstrated. Selected genes are being tested in plant systems and will be discussed.

1.4.1. The 'giant genus' problem: issues, solutions, opportunities?

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Giant genera comprise up to 20% of all vascular species and are often poorly known systematically. Why do we have giant genera?: (1) historical artifact of the genus concept in ranked systems of classification; (2) recognition of paraphyletic genera using symplesiomorphic features (e.g., *Psychotria*); (3) failure to identify polyphyletic genera exhibiting convergent or parallel apomorphies (e.g., *Salvia*); and (4) extensive radiation in one monophyletic lineage through a "key innovation" of an apomorphic feature (e.g., *Ficus*, *Carex*). Future systematic studies in giant genera should include collaborative efforts directed in a multi-disciplinary fashion (e.g., *Croton*, *Solanum*, *Salvia*). A "top down" survey approach will be crucial prior to more detailed "bottom up" studies. Web-based dissemination of systematic knowledge will be necessary rather than reliance only on the career-long and often unpublished "big picture." Necessary nomenclatural changes must be explored in a manner that best serves the systematic community and the larger "user" community of systematic knowledge.

1.4.2. Big plant genera: emergence, growth, neglect and renewal

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That higher plant genera were not uniform in size was early apparent, and indeed the sheer diversity of some soon attracted connoisseurs. However, only around 1900 - with more extensive records - was their size distribution seen as exponential. The 28 with 300 or more species were first listed by N.E. Brown in 1883. Further lists by J.C. Willis and R. Good of genera with 500 or more followed by 1960. Yet these 'giants' were but one end of a Zipf curve; at the other were monotypes - and in statistical terms there was no distinction. Some botanists, however, believed big genera to embody more than mere numbers: there might also be keys to a better understanding of plant palaeohistory, evolution, and comparative diversification. Only recently has this challenge been taken up, notably through collaborative effort with approaches from alpha taxonomy to genomic studies and cladistic analyses of relationships and probable phylogeny. I shall here explore the sequence and circumstances of, and approaches to, their documentation (and initial subdivision) prior to the mid-twentieth century, factors leading to their neglect, the faith of their proponents, and recent developments.

1.4.3. Many *Acacia* species, little genetic variation: DNA sequencing and the use of the microarray platform for inferring relationships among closely related species

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Acacia contains over 1,500 species mostly found in arid-zones worldwide. Chloroplast DNA sequence analysis has determined the existence of at least 5 lineages which are distributed within the tribes Ingeae and Mimoseae. These lineages conform to established subgenera (*Acacia* and *Phyllodineae*) while a third subgenus is split into three lineages. Within most lineages sequence variation is sufficient to study relationships, biogeography and character evolution hypotheses. However, relationships among the 950 Australian species (subg. *Phyllodineae*) are not well resolved by DNA sequencing. A genotyping technique using the microarray platform, DaRT (Diversity Arrays Technology, DaRT Pty Ltd), is being used to survey genetic relationships within this group. The procedure digests bulked DNA from target species, clones the fragments and spots them on slides using standard microarray protocols. Digested DNA from an individual is hybridized to the slide yielding data of the presence or absence of thousands of restriction fragments spotted on the slide. These data are compared to DNA sequence data in their ability to uncover phylogenetic relationships.

1.4.4. Euphorbiaceae s.s., a constellation of giant genera

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Even when considered in the strict sense, Euphorbiaceae contains three giant genera - Euphorbia, Croton, and Acalypha that together total over 3,500 species (well over 1% of all flowering plants). Euphorbia has always been considered a large genus, but its circumscription has been open to various interpretations. An inclusive view of the genus would group all of the cyathial inflorescences, which may be a key to the great diversification of the clade. Molecular sequence data supports Croton as being largely monophyletic, with just one clade of 12 species (*Astraea*) split into a distinct clade, and many segregate genera included with Croton, such as *Eremocarpus*, *Julocroton*, *Moacroton*, and *Cubacroton*. With appropriate sampling and use of molecular data, most of the 40 sections recognized by Webster in 1993 can be evaluated for monophyly and intersectional relationships. Many of the circumscriptions of Old World sections do not hold up, whereas many of the New World sections are strongly monophyletic or can at least be redefined. Pollination mechanisms and ecological roles in the three large genera will be compared as a possible key to their diversifications.

1.4.5. Taxonomy as a team sport: PBI *Solanum* or how to monograph a monster

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Solanum (Solanaceae), with between 1500 and 2000 species, ranks among the ten most species-rich genera of flowering plants, and is a truly large, monophyletic genus. Unlike some other large plant genera, molecular phylogenetic work has supported its monophyly. Taxonomic recognition of such groups must be a mix between pragmatism - ease of recognition for non-botanists is an often over-looked criterion - and perfection. Global taxonomic revision of such large, monophyletic groups is impossible for a single botanist, given the sheer volume of literature, specimens and techniques available today. We will explore how we are approaching a global species-level revision of *Solanum*. Both scientific and sociological issues can impede progress. Harnessing the power of the Internet and presenting data electronically only goes some way to overcoming the barriers to tackling such a group. Cooperation of large groups of taxonomists working towards common, rather than competitive goals, is key for monographing species-rich genera whose monophyly and ease of recognition makes their treatment as a whole logically preferable.

1.4.6. Are there so many species of *Astragalus*? a repris

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Astragalus (Leguminosae) is considered the most species-rich angiosperm genus. We explore the implications of this claim, beginning with a discussion of the interplay between taxonomy and diversification rate, then revisit previous efforts to reconstruct diversification in *Astragalus* in light of recent molecular phylogenies and divergence time estimates, and better analytical tools than available a few years ago. Two complementary approaches include estimating net diversification rate based on extant diversity and estimated crown group age of the clade, and using the more powerful Kendall-Moran estimator of rate that is based on all the available node times within a clade. We separately estimate rates for a nested set of clades including the larger Astragalean clade, *Astragalus*, and Neo-Astragalus, with an analysis of confidence limits on the diversification rates that includes consideration of the errors introduced into divergence time estimation. Finally, to place diversification in *Astragalus* in a broader phylogenetic context, we compare estimates of diversification rates in several other legume genera, such as *Indigofera*, *Acacia*, and *Lotus*.

1.4.7. Treelike and reticulate phylogenetic history of Sileneae and its implications on taxonomy

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A problem for taxonomic research of species-rich groups is the availability of information itself. This is a bioinformatics problem that has not received the attention it deserves. A classic problem is delays in publication of revisions, due to the strive to be "complete". We suggest that this can be solved with a database-based approach. Another problem for hierarchical taxonomy is violations of the tree-like model. To account for this there is a need to study multiple, potentially unlinked gene phylogenies. For the 700+ species in Sileneae, we have chosen the rps16 intron and the psbE/petL spacer from the chloroplast genome, the nuclear ribosomal ITS regions and intron regions from the nuclear low-copy number genes RPA2 and RPB2 as "standard" sequence regions. This serves as hypothesis-generating, and - for specific problems - we add other regions depending on the problem. We exemplify this approach on problems pertaining to homoploid hybridization, allopolyploidy and very short internal branches with the hexaploid *Silene hookeri*, which has rather remotely related parent lineages.

1.5.1. The evolution of the Cape flora as a model system

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The Cape flora is centered in the southern tip of Africa, and is characterised by some 30 clades which have their phylogenetically basal elements and greatest species diversity in the Cape Floristic Region. This flora is remarkably species rich, with numerous closely related species concentrated in a geographically restricted area. Current indications are that many of these clades have been in southern Africa since the mid-Tertiary, but that there was no single trigger for the diversification of the modern species flocks. The oldest radiation documented to date is that of the Restionaceae, which was initiated in the Oligocene. The modern species diversity of the flora may date from the Plio-Pleistocene. This fits the current model of initial climatic deterioration in the Oligocene, followed by a mesic Early Miocene, and then accelerating deterioration after 14 Mya, probably associated with increased summer-drought in south-western Africa. The modern richness might be more the result of little extinction, rather than recent and rapid radiation.

1.5.2. Geographical affinities of the Cape flora

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The flora characteristic of the Cape Floristic Region (CFR) is dominated by a relatively small number of clades that have been proposed as 'Cape clades'. These clades have variously been suggested to have African or Austral affinities. Here we evaluate the support for these hypotheses using data from phylogenetic analyses. We further test the hypothesis that these clades share a common time of differentiation from their geographical neighbours. Many Cape clades show Austral rather than African relationships and relatively few Cape clades show a sister-relationship to South America and tropical Africa, despite their relative geographical proximity. These and other numerous patterns are suggestive of a cosmopolitan flora. There is no simple hypothesis that can account for the geographical sources of the currently distinctive Cape flora. This spatial variation is echoed in the temporal data; although there is wide variance around the dates of disjunctions, it seems that the Cape flora has been assembled over a long time period.

1.5.3. Growth-environment-driven divergence in the Cape flora

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A significant proportion of Cape species diversity is attributed to the radiation in vegetative characters exhibited by several extremely species-rich Cape Clades (clades which have their

phylogenetically basal elements and greatest species diversity in the Cape Floristic Region). Two case studies of vegetative divergence in Cape Clades are explored here. The first examines a remarkable case of vegetative convergence (between *Aspalathus*, Legumionosae and *Cliffortia*, Rosaceae) which may retain the footprint of environmental change. The hypothesis that the radiations are contemporaneous is explored here. The second considers the a novel dispersal mechanism (fleshy fruit) in genus *Muraltia* (Polygalaceae) which may act as a key innovation driving vegetative adaptation. Morphological diversity, environmental amplitude and geographic distance are compared for a fleshy-fruited clade and its dry-fruited sister clade. The fleshy-fruited clade shows greater diversity by all three measures.

1.5.4. Can we reject pollinator-driven speciation as a predominant model for the Cape Floristic region?

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Many lines of evidence point towards a recent burst in speciation in a few large lineages in the Cape Floristic Region. This begs the question as to what was the main mechanism that generated this biodiversity. Historically, two competing hypotheses have been put forward to explain this pattern. Either adaptation of diverging conspecific populations to the ecologically heterogeneous environment was the main factor driving speciation, with adaptation to different pollinators only being necessary upon secondary contact to protect species integrity. Alternatively, adaption to different pollinators was a primary factor driving speciation. To test these, we review all the available evidence by combining phylogenies, ecological data, distribution data and pollinator data of lineages centered in the Cape Floristic Region. Support for either hypothesis is provided by comparing the degree of sympatry, ecological divergence and pollination mode between sister species pairs.

1.5.5. Floral and vegetative evolution in Cape pelargonium (Geraniaceae): fast but uncoupled

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Pelargonium (Geraniaceae) ranks in the top ten Cape Floral Region genera, with 148 species found within the CFR. Pelargonium consists of five majorclades, structured in two main groups that correlate with chromosome size. A wide range of variation in morphology and life form is found in one large clade, comprising almost 60% of the total number of species known. This Cape 'winter rainfall' clade harbours, apart from many evergreen shrubs, a large xerophytic clade comprising geophytes, stem and leaf succulents. Nested within this is an exclusively geophytic clade, comprising c. 80 species of sect. *Hoarea*, characterised by the possession of tunicate tubers, and possibly representing a non-adaptive radiation. Speciation in this clade appears to be predominantly allopatric and pollinator-driven. Nectar spurs have enabled Pelargonium to use a wide array of pollinators, notably long-tongue hovering flies and hawkmoths, a pattern that appears to be repeated within the geophytic *Hoarea* clade. Overall, we conclude that evolution of geophytism shows highest correlation with increase in clade-size in Pelargonium and that floral evolution occurs at a rapid rate.

1.5.6. Leaf shape evolution in Pelargonium

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The plant genus *Pelargonium* L' Hér. (Geraniaceae) has undergone an extraordinary South African radiation in growth form and leaf shape. Growth form, which is roughly correlated with sectional classification, ranges from an annual habit, through geophytes, stem succulents and woody shrubs. There also exists remarkable the diversity in leaf shape (which is uncorrelated with growth form) that includes variation in the extent of dissection, leaf size and venation patterns. We scored over one hundred species for traditional shape characteristics (apex, base, outline, margin,

degree of dissection and patterns of major veins). We then reconstructed ancestral character states using parsimony for branches leading to major clades. All major clade branches derive from an ancestral state of: rounded apices, cordate bases, and ovate outlines, with palmate major veins. A shift to pinnate/intermediate leaf venation occurs along the branch leading to the winter rainfall clade. The degree of dissection of the lamina showed no resolution at any of the major basal branches, suggesting that dissection reflects species-specific adaptations within clades.

1.5.7. Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape flora of Southern Africa

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Heliophila, *Cycloptychis*, *Thlaspeocarpa*, *Schlechteria*, *Silicularia*, *Brachycarpea*, and *Chamira* are endemic genera in the Cape region of South Africa and they are regarded as the most diversified Brassicaceae in every aspect of habit, leaf, flower, and fruit morphology. Relationships and taxonomic limits among these genera are unsettled. Our ITS study demonstrates monophyly of these genera, excluding *Chamira*. The molecular analysis reveals parallel evolution of fruit characters, typically used in the traditional classification schemes. The recognition of three main clades corresponds largely with the distribution of morphological characters (e.g., habit, leaf shape, etc.) not adequately accounted for in previous studies. Estimation of divergence times of the main lineages of *Heliophila* is in agreement with recent analyses in other plant groups, all of which date the diversification against a background of aridification in the Pliocene/Pleistocene. Ecogeographical evolution and the adaptive significance of various floral structures is discussed in terms of their possible role in the rapid diversification within *Heliophila*.

1.6.1. Introduction to polyploid genome evolution and lessons from *Gossypium*

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Polyploidy is a prominent process generating a great deal of biodiversity. Recent years have witnessed many advances in our understanding of the novel genic and genomic interactions that accompany genome doubling in many plant groups, as encapsulated in this symposium. In *Gossypium*, there have been multiple episodes of trans-oceanic dispersal, some leading to hybridization among lineages that presently are inter-sterile. Allopolyploid *Gossypium* arose from an intercontinental merger 1-2 million years ago of two genomes that evolved in isolation for 5-10 MY. This remarkable biological reunion led to a spectrum of genic and genomic interactions, including concerted evolution, intergenomic transfer, a high frequency of gene silencing and possible functional differentiation among homoeologs. Comparisons of synthetic and natural polyploids demonstrate the scope of epigenetic silencing accompanying polyploid formation and the pace of subsequent expression evolution. Microarray experiments are being used to explore gene expression patterns in polyploids and the possibility of novel gene recruitment in certain pathways following genome doubling.

1.6.2. Precise polyploidy-related evolution mechanisms in wheat (*Triticum* and *Aegilops*) species

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Although polyploidy has been shown to induce important genetic and epigenetic events, such as DNA sequence elimination, nothing is yet known about their molecular mechanisms. We investigated the molecular basis of such evolution mechanisms by comparing, at several selected loci, corresponding sequences of diploid, tetraploid and hexaploid wheat species (*Triticum* and *Aegilops*). The comparative analysis allowed us to determine the sequences of the rearranged regions as well as rearrangement breakpoints. Among these genomic rearrangements, the previously reported *Pina* and *Pinb* genes loss from the important *Hardness* locus of polyploid wheat species was shown to be caused by a large genomic deletion, through illegitimate recombination, which

occurred independently in the A and B genomes. Various illegitimate DNA recombination events that lead to genomic deletions, duplications and inversions were determined and appear to constitute the major evolutionary mechanisms that accompanied tetraploid and hexaploid wheats formation. This work represents the first report on precise molecular basis of polyploidy-related evolution mechanisms.

1.6.3. Recent allopolyploidy and genome evolution in *Spartina* (Poaceae)

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The evolutionary and genomic history of polyploid *Spartina* species will be reviewed, with a special interest in the role of reticulate evolution and genome duplication in the success of newly formed hybrid and allopolyploid lineages. *Spartina* species are tetraploid, hexaploid or dodecaploid perennials, most of them native to the New World. Recent (19th century) hybridisation and polyploidisation events involved hexaploid species, following introduction of the east-American *S. alterniflora* into Western Europe. Two independent hybridisation events with the Euro-African *S. maritima* resulted in hybrids that differ dramatically in morphology, i.e., *S. x neyrautii* (in France) and *S. x townsendii* (in England), both with *S. alterniflora* as the maternal parent. Chromosome doubling of *S. x townsendii* resulted in a particularly successful new allopolyploid species, *S. anglica*, which has rapidly expanded in range and has now invaded several continents. We use this system to explore the genetic, epigenetic and expression dynamics of recent hybrids and allopolyploid taxa in Western Europe.

1.6.4. Genomic and transcriptomic changes during and following allopolyploid speciation in *Senecio*

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The discovery of new allopolyploid species in the wild provides an opportunity to examine what changes in genome structure and gene expression accompany allopolyploid speciation. The Welsh groundsel, *Senecio cambrensis*, is one of five new allopolyploid species known to have originated within the past 100 years or so. It originated independently at two different sites in the United Kingdom following hybridization between diploid *S. squalidus* and tetraploid *S. vulgaris*. We have found that the Welsh lineage of this allohexaploid species contains high levels of AFLP genotypic diversity indicating that intergenomic recombination is common. A genomic map of *S. cambrensis* has been constructed for comparison with maps of the parent species to assess levels of intergenomic exchange. In addition, we have developed and used an anonymous cDNA microarray to examine genome-wide alterations to gene expression between parents, the triploid hybrid and the allohexaploid using wild and synthetic material. Dramatic differences in expression were recorded between the four taxa demonstrating the impact of hybridization and polyploidy on gene expression in higher plants.

1.6.5. The genetic consequences of polyploidy in *Nicotiana*

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Here we report on the genetic consequence of polyploidy from ancient to recent in *Nicotiana*. Polyploidy has been associated with a number of genetic events, these include: (i) homogenisation of rDNA; (ii) transcription silencing of rDNA; (iii) loss and gain of DNA sequence; (iii) stabilising chromosome translocations; (iv) genome “downsizing” and (v) rapid evolution of paternal genomes. These phenomena are studied using recently made synthetic *Nicotiana* allotetraploids. Data are compared with natural polyploids from *Nicotiana* sections of widely different ages and hence periods of time for genetic divergence (thousands to million of years). We find rapid changes in the first few generations, these include the

amplification and spread of a novel rDNA family across and between loci and the loss of tandem and dispersed repeats. Thereafter, over long-time scales (>20,000 years old) we observe genome diploidisation processes including the divergence of repetitive sequences and the loss of rDNA loci.

1.6.6. Genetic and genomic consequences of recent and recurrent allopolyploidy in *Tragopogon* Asteraceae

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Tragopogon mirus and *T. miscellus* are allopolyploids that formed repeatedly during the past 80 years following the introduction of three diploids (*T. dubius*, *T. pratensis*, and *T. porrifolius*) from Europe to western North America. Because the parentage and time of formation of these polyploids are well documented, this system provides a unique opportunity to study the consequences of recent and recurrent polyploidization. We summarize the cytogenetic and genomic aspects of polyploidy in *Tragopogon*, emphasizing our recent studies of gene expression in the newly formed allopolyploids. Specifically, cDNA-AFLPs reveal differences in gene expression between *T. miscellus* and its diploid parents, as well as between populations of *T. miscellus* of reciprocal origin. These differences include novel expression and gene silencing, with some patterns of gene silencing corresponding to maternal and paternal effects. The recent generation of artificial F₁ hybrids between the diploid progenitors will allow us to explore the impact of hybridization versus polyploidization on the genomes of the tetraploid species.

1.6.7. Independent ancient polyploidy events in the sister families Brassicaceae and Capparaceae

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Recent studies have elucidated the ancient polyploid history of the *Arabidopsis thaliana* (Brassicaceae) genome. The studies concur that there was at least one polyploidy event, identifiable by numerous duplicated gene pairs in syntenic blocks, which occurred some 20 to 60 mya and near the divergence of the Brassicaceae from its sister family, the Capparaceae. Using a comparative genomics approach, we have asked the question of whether this polyploidy event was unique to members of the Brassicaceae or if it was shared with the Capparaceae. We have isolated and sequenced three genomic regions from diploid *Cleome spinosa* (Capparaceae) that are homologous to a duplicated region found on At3 and At5. The three genomic regions in *Cleome* are each unique and are mosaics of At3 and At5 specific genes, with no clear orthology/paralogy gene pair relationships. Genes found in duplicate in *A. thaliana* also tend to be replicated in *Cleome*. Surprisingly, molecular evolutionary analyses found that the triplication in *Cleome* occurred more recently, and independently, of the duplication event in *A. thaliana*, but that the rate of gene loss is higher in *Cleome*.

1.7.1. Research on *Orobanch*: what do we know and what should we get to know?

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Orobanch spp. (broomrapes) are parasitic plants that develop multicellular haustoria, which connect to the roots of various dicots. Their tiny seeds germinate only after perceiving a host stimulus. Some species are weedy and cause severe damage to agriculture. The globalization of these parasitic weeds, the evolution of new races, and the gradual increase in their virulence pose threat to many crops worldwide, while means to control them are scarce. Studies of key developmental mechanisms of the parasitic interaction, regarding germination, attachment, haustorium penetration and host resistance, are needed for the understanding of their evolution. Proteomics and genomics should provide data on the genes involved in host-parasite interaction. Manipulation of developmental and metabolic pathways, which are involved in the parasitic interaction in both host and parasite, may provide protection against parasitic weeds. New sources for resistance in host plants may also be developed, employing specific antibodies, gene silencing, local release of toxins, and false signals.

1.7.2. Molecular phylogenetics and taxonomy of *Orobanches*. I. (Orobanchaceae)

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Phylogenetic analyses of DNA-sequence data have greatly changed our understanding of circumscription of Scrophulariaceae and allied families, e.g., Orobanchaceae, traditionally comprising exclusively holoparasitic taxa, now include both hemi- and holoparasitic members. In a similar way, recent molecular phylogenetic analyses suggest substantial re-definition of the genus *Orobanche*, which is the largest of the holoparasitic genera in Orobanchaceae. One group, with chromosome base number of $x = 12$, contains the each monophyletic sections *Gymnocaulis*, *Myzorrhiza*, and *Trionychon*. The second group, with chromosome base number of $x = 19$, contains *Diphelypaea* and *O. sect. Orobanche*, the latter again comprising three distinct lineages. The relationships between these two major groups and those to other genera are unclear. As already suggested by previous authors, recognition of the sections of *Orobanche* as separate genera would allow to better reflect the phylogenetic patterns in a taxonomic system.

1.7.3. *Orobanche*-host plant interactions: ecological and physiological aspects

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Among the various *Orobanche* species, a large difference in host range exists from very small to extremely wide. Because the *Orobanche* lifecycle is highly specialized for parasitism, the first stages of host-parasitic interactions seemed to be essential in determining host specificity. *Orobanche* seed germination is dependent on several abiotic and biotic factors as shown in various ecological and physiological studies. Although this first stage can be considered as the most discriminating one in terms of host selection, the germination process is too complex to draw definitive conclusions. The next stages crucial for the development of *Orobanche* are haustorium initiation, host tissue penetration and physiological compatibility. Progress in understanding these processes have been made in recent years using host plant-parasite model systems. After successful infection *Orobanche* relies completely on its host for the supply of both reduced carbon, nitrogen and also minerals. The response of the host to infection by the holoparasite can be explained by just source-sink relations and will be discussed with respect to other parasitic angiospermhost systems.

1.7.4. Predictive empirical modelling of *Orobanche* life cycle and seed ecology

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Dormancy in *Orobanche* seeds is removed as a two-stage process. First, after shedding from the mother plant, populations of dry *Orobanche* seeds after-ripen (lose primary dormancy). Increasing the temperature results in a faster after-ripening rate of the seed population. The second stage - conditioning - occurs at water potentials close to full imbibition. The rate of conditioning in the seed population also increases with increase of temperature. Secondary or even tertiary processes may interfere with dormancy loss. For example, prolonging the conditioning period beyond about 21 days decreases the subsequent germination response due to a) induction of secondary dormancy and b) at temperatures over about 25°C, loss of viability. Germination of conditioned seeds depends on chemical stimulation from host's roots, temperature and water stress. The thermal time for germination is strongly affected by water potential. Non-linear probability models account for these processes and provide predictive empirical models to describe the germination behaviour of the seed population. The impact of these results is discussed for *O. aegyptiaca* parasitising tomato.

1.7.5. Population genetics in *Orobanche* species

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The studies of population genetics of *Orobanche* species are of great importance since the understanding of the variability within and between pathogenic populations is essential if selection programmes need to target sources of resistance in different areas and suitable breeding strategies need to be developed. The use of morphological markers in *Orobanche* has been difficult because holoparasitism resulted in a reduced number of suitable characters even for taxonomical purposes. The distribution of genetic variation in *O. aegyptiaca*, *O. crenata*, *O. cumana*, *O. foetida* and *O. ramosa* populations has been reviewed based on available molecular marker data. It is shown that by using simple DNA marker systems (e.g. RAPDs and ISSRs) the intraspecific variability can be assessed and problematic issues such as the differentiation between botanical taxa, geographical differentiation, and host-differentiation can be addressed.

1.7.6. Evolution of Ty1-copia and Ty3-gypsy retroelements in holoparasitic *Orobanche* (Orobanchaceae)

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Retrotransposons (RTNs) are mobile genetic elements, which transpose via an RNA intermediate. The transposition of RTNs is replicative, i.e., the existing elements are not excised but the newly formed copies re-integrate into new positions in the host genome. This feature, among others, such as ubiquity and high copy number, renders RTNs excellent molecular markers. We amplified and sequenced *rt* gene of Ty1-copia and Ty3-gypsy RTNs (at least 20 clones for each RTN type) from 13 *Orobanche* accessions. These accessions are (i) the economically most important weeds *O. aegyptiaca* and *O. ramosa* of sect. *Trionychon* and *O. cernua* and *O. crenata* of sect. *Orobanche*, and (ii) one close wild relative of each of these four taxa. While in sect. *Trionychon* RTN diversity is higher with many small unique RTN families, fewer RTN families are found in sect. *Orobanche*, some of them comprising numerous and very similar *rt* sequences. This suggests that species in sect. *Orobanche* might have experienced a recent burst of RTN activity. Additionally, we found indication of horizontal gene transfer for Ty1-copia and Ty3-gypsy RTNs, both from sect. *Trionychon* to sect. *Orobanche* and vice versa.

1.7.7. *Orobanche* species as weeds and their management in sustainable agriculture

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About 3500 flowering plant species have lost their autotrophic way of life during evolution and parasitise other plants in order to supply themselves with water and nutrients. They are more than a fascinating group of plants as a few of them are severe constraints to major crops. By far the most economically damaging parasitic weeds are members of the genera *Striga* (witchweeds) and *Orobanche* (broomrapes). The most damaging *Orobanche* species are *O. crenata* that causes huge damage to legume crops, *O. cumana* that threatens sunflower, *O. ramosa* that attacks potato, tobacco, tomato and hemp, and *O. aegyptiaca* has very severe effects on legumes and vegetables, but also other species might gain importance. The spread of these parasitic weeds, and the gradual increase in their virulence pose threat to many crops worldwide, while means to control them are scarce. In the present work we revise their importance and re-evaluate control methods in the light of recent developments in crop breeding and molecular genetics.

1.8.1. Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: evolution of a versatile fungal genus

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Trichoderma/Hypocrea is a genus of soil-borne or wood-decaying fungi, which can make up a high portion of fungal propagules in soil. It contains members that are important to mankind as producers of industrial enzymes and biocontrol agents against plant pathogens, but also as opportunistic pathogens of immunocompromised humans. Most recently, endophytic

Trichoderma spp. have also been found. Species identification, while essential in view of the controversial properties of taxa of this genus, has been problematic by traditional methods, therefore rendering the species identity of isolates studied in older literature uncertain. In this lecture, I will demonstrate how the Genealogical Concordance Phylogenetic Species Recognition Concept (GCPSR), when combined with Bayesian models of evolution, provides insights into the biodiversity, modes of speciation and evolution of ecological characters in this ubiquitous fungal genus.

1.8.2. New aspects on ecology and biodiversity of the Sebaciales (Hymenomycetes, Basidiomycota), plant associates with a uniquely wide spectrum of mycorrhizal interaction types

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Within the basidiomycetes, the vast majority of known mycorrhizal species belong to homobasidiomycetes. It was therefore surprising that during the past years molecular and ultrastructural studies revealed a broad diversity of mycorrhizal associations involving members of the new heterobasidiomycetous order Sebaciales. It became evident that members of this order are involved in a wide spectrum of mycorrhizal types: ectomycorrhizas, orchid mycorrhizas (both with green and myco-heterotrophic orchids), ericoid mycorrhizas, and also in recently described associations with some members of the liverworts (Jungermanniales) that resemble mycorrhizas at the cellular level ('jungermannioid mycorrhizas'). A comparably broad diversity of mycorrhizal associations is known from no other fungal group. We give an overview over the present knowledge concerning phylogeny, morphology and ecology of the Sebaciales, particularly focussing on new results concerning associations with Ericaceae.

1.8.3. Molecular systematics of the Zygomycetes: organismal diversity, spore morphology and a multi-gene based phylogeny

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The Zygomycetes encompass microscopic fungi which form zygospores in sexual interactions. The most prominent and largest orders are the Mucorales and the Mortierellales, comprising in most cases saprotrophic but also facultatively parasitic species, which occupy a large variety of ecological niches. We investigated zygomycetous soil fungi from different natural habitats, e.g. from meadow land among the Danube river, alpine territories, domestic areas and forest biotopes. The goal was to assess the genotypic diversity in correlation with the phylogeny and systematics. To this end we generated multigene genealogies in conjunction with physiological and detailed sporangiospore morphology obtained by scanning electron microscopy. Phylogenetic analysis in the context of available sequence data (approx. 6,000 nucleotide positions per species) strongly supports sporangiospore morphology and thermophilic growth as suitable distinguishing characters. Moreover, suggestions of a revised family nomenclature in zygomycete systematics will be discussed.

1.8.4. Conflicting evidences about the phylogeny of ascomycetous yeasts estimated by 18S rDNA sequences and cell wall carbohydrate composition

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Small subunit rRNA gene sequences (18S), urease-activity and cell wall carbohydrate composition were analysed within a larger number of ascomycetous yeasts and filamentous fungi to validate their congruence in predicting phylogenetic relationships. Three

carbohydrate patterns, glucose-mannose, mannose-glucose-galactose-rhamnose and glucose-mannose-galactose-rhamnose-(fucose), characterise the Hemiascomycetes, the Euascomycetes and the Protomycetes, respectively. In addition, the glucose-mannose-galactose carbohydrate profile can be found in representatives of all the three classes. Chemotaxonomic studies coupled with additional cytological and co-evolution studies support the idea that the Hemiascomycetes occupy a basal position in the phylogeny of Ascomycota. These results are not in line with the phylogenetic studies based on the sequences of 18S rRNA encoding gen. The maximum parsimony analysis indicated that Hemiascomycetes and Protomycetes might represent sister groups. The term Protomycetes was introduced reflecting much better the properties of the whole class.

1.8.5. New anamorphic Basidiomycota filling gaps in fungal diversity studies

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Anamorphic Basidiomycota provide essential data for understanding the life-cycle (saprobic stage vs. biotrophic stage), ecology (geographic distribution, substrates, vectors etc.), and systematics (morphological, molecular, and other characteristics) as well as the general diversity of the Basidiomycota. In this contribution, this significance of anamorphs is illustrated with examples of new anamorphic taxa (hyphomycetes and yeasts) connected with the Cryptomycocolacales (*Colacosiphon*), Atractiellales (*Leucogloea*), Agaricostilbomycetidae (*Kurtzmanomyces*), Dacrymycetales (*Dacryoscyphus*), Auriculariales (*Helicomysa*), Tremellales (*Sterigmatosporidium*), as well as the Agaricales (anamorphic *Marasmius*) and other homobasidiomycetes (*Cruciger*). The new taxa are based on morphological, ultrastructural, physiological, and molecular findings in collections made in Middle Europe, China, and Taiwan.

1.8.6. Utility of chemotaxonomy for assessment of phylogenetic relationships and generic concepts within the Xylariaceae

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The biological and chemical diversity of *Xylariaceae* with emphasis on genera with *Nodulisporium*-like anamorphs (e.g., *Daldinia*, *Hypoxyton*) was evaluated by a HPLC-based chemotaxonomic survey of ca. 2000 specimens and cultures, accompanied by extensive morphological studies and SEM. Conspecificity of recent records with old type specimens by comparison of HPLC profiles was safely established in many cases, since the characteristic metabolites may remain stable for over 200 years. Several of them constitute novel natural products that were identified from stromata of *Xylariaceae* in the course of concurrent "mycochemical" studies. These results were found largely in agreement with those of concurrent molecular studies, involving 5.8S/ITS nrDNA sequences. While anamorphic morphology and metabolism of cultures agreed well at generic level and above, a combination of chemical and morphological traits appears suited better than even molecular data for differentiation of these fungi at subgeneric level. A polythetic approach is most likely to result in a stable phylogeny of these fungi, as well as for attempts to estimate their biological diversity.

1.8.7. Tuberculina "Helicobasidium: cheating fungi" killing plants

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Helicobasidium species (Urediniomycetes) are serious plant pathogens causing the economically important violet root rot. In its sterile stage, the fungus is known as *Rhizoctonia crocorum* or *Thanatophytm*, respectively. In contrast, *Tuberculina* species are mycoparasites of rust fungi. The strong inhibitory effect of *Tuberculina* on rust spore production has resulted in extensive research dealing with *Tuberculina* as a biological agent in rust control. Recently, we demonstrated that rust-parasitic *Tuberculina* species and plant-parasitic *Helicobasidium* species are stages of the life cycle of one holomorph. Combining the results of infection experiments, molecular analyses, and morphological and ecological characters, we show that there are at least six monophyletic lineages within the *Tuberculina*/*Helicobasidium*-

group that can be unambiguously distinguished. The thereby revealed diversity opens up new perspectives on the evolution of this exceptional mycoparasitic-phytoparasitic fungal group.

1.9.1. Ecological speciation in an East Maui endemic *Dubautia* (Asteraceae) species flock

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The process of adaptive radiation has been the subject of considerable debate. Several hypotheses have been proposed to explain the rapid diversification of taxa into varied habitats. The ecological theory of adaptive radiation postulates that divergent natural selection drives rapid speciation and phenotypic diversification. One recently evolved example of a monophyletic species radiation into a small species flock is the East Maui endemic *Dubautias*: *D. menziesii*, *D. platyphylla*, *D. reticulata*, and *D. waiianapanapaensis*. Despite the close genetic relationships, these species are morphologically and ecologically distinct. These four species, therefore, represent a recent ecological diversification into different niches, apparently based upon fine-level partitioning of ecological habitats. They are capable of interbreeding where they come into contact, but typically inhabit different habitats and maintain their morphological distinctness. Here, we examine variation within and among populations of the East Maui endemic species of *Dubautia* using microsatellite, DNA sequence, leaf morphometric, and ecological data.

1.9.2. Current Trends in Western Balkan *Campanula* L. Research

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Genus *Campanula* is traditionally considered to be very complicated from a taxonomic point of view, due to its phenotypic plasticity, ontogenetic variation and polyploidy. Flora of the Western Balkans comprises up to 100 *Campanula* taxa, out of which more than 30% endemic. Mutual relations of these taxa puzzle taxonomists for centuries: today, using comparative morphological, chorological and ecological data, in combination with the results of extensive cytological, isoenzyme and molecular (chloroplast and nuclear DNA) approaches, we are closer than ever to finally understand the obscure relationships among the Balkan-*Campanula* taxa lineages, and to other campanuloids, their ways of surviving the Ice Ages and possible paths of re-colonisation, followed by fast evolution of taxa. Several indigenous endemic lineages (*Isophylla*, *Waldsteiniana*, *Pyramidalis*, *Rotundifolia*) are discussed closely, to demonstrate promising comparative approaches in solving mutual relations inside this perplex genus.

1.9.3. Large scale AFLP screening of natural populations provides the basis for the analysis of the molecular mechanisms underlying biodiversity - the case of *Melampyrum* (Orobanchaceae)

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Analyses of mutants and "ecotypes" using mainly seedbank material cover only a small part of the variation present in natural populations. By contrast, in nature there is a large range of variation, encompassing an unknown number of distinct ecotypes, varieties and mutants. Within the 6 species of *Melampyrum* known in Trentino/Italy, AFLP analyses revealed considerable genetic distances within and between populations as well as differences between species concerning population- and meta-population structure. Beside several morphological and ecological traits varying between populations and species also a flower-mutant independently appearing in different populations was discovered. Furthermore there is a lack of correlation between morphological and genetic distances between the species to the point that even a 7th species was found. While genomic-based AFLP provides the basis to define the populations or species to be compared, other techniques, like cDNA-AFLP are used to identify the genes involved in the traits analyzed. A summary of our approach to trace the molecular mechanisms underlying biodiversity by analysing natural populations is given.

1.9.4. Within-population genetic variation of the distylous *Primula veris*: does morph ratio matter in fragmented habitats?

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In Flanders (northern Belgium), *Primula veris* (Primulaceae), a distylous self-incompatible perennial herb, mainly occurs in fragmented habitats of the intensively used agricultural landscape. We investigated within-population and within-morph genetic variation and structure for 30 allozyme loci in 24 populations of *P. veris* from two distant regions (Westhoek and Voeren), in relation to population morph bias and morph frequencies. Enzyme polymorphisms indicate that the Wright's inbreeding coefficient (F_{IS}) values were negatively correlated to the proportion of pin individuals in the population. Three populations showed a significant genetic differentiation between pin and thrum morphs. Contrasting fine-scale spatial genetic structure patterns were found for pin and thrum morphs. The results are discussed in the light of demographic stochasticity, morph-specific differences in self-compatibility levels and reproductive fitness, historical factors (bottlenecks), and pollination patterns.

1.9.5. Deleterious mutation in plant species with contrasting mating systems

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The genus *Amsinckia* (Boraginaceae) is a group of western North American annual plants that exhibit remarkable interspecific and intraspecific variation in the rate of self-pollination, variation that is closely tied to the presence of distyly in populations. Phylogenetic studies of this group suggest that extant homostylous and distylous populations are often very closely related. Here we present the results of studies designed to estimate deleterious mutation parameters in two closely related species of the genus with contrasting mating systems. The results show there are no marked differences in the rates and effects of deleterious mutation affecting a major fitness component, flower number per plant, implying that mutation rate does not evolve with the mating system. This observation may help account for the apparent pattern of recent mating system diversification within this plant group.

1.9.6. Evolution of herbivore-induced resistance in wild mustard (*Sinapis arvensis*)

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Induced responses of plants to herbivore feeding play an important ecological role in plant-herbivore interactions. Several chemical, morphological, and life-history traits can be induced and their inducibility may be under selection by herbivores. An evolutionary response to selection may be constrained by a lack of genetic variation or by negative genetic correlations. We used a quantitative genetic approach with controlled crosses, that allowed the estimation of additive genetic variances and covariances. In an experiment the responses of *Sinapis arvensis* to feeding by a specialist herbivore, *Pieris rapae*, were investigated. We measured a number of resistance traits and performed bioassays to determine resistance against *Mamestra brassicae*, *Spodoptera littoralis*, and *Pieris rapae*. We present estimates of the heritability of constitutive and induced resistance traits, and explore the pattern of genetic correlations among these traits in order to predict their evolutionary potential.

1.9.7. The effects of selection on a polyploid series

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The development of an array of molecular markers in the last twenty years has enabled evolutionary relationships between taxa to be precisely determined. Consequently a large number of such studies have been carried out, focussing on such modes of speciation as progenitor-derivative pairs and polyploid series. At the same time the effects of natural selection have been less likely to be investigated, although this was the predominant area of research in the early days of ecological genetics. Where evolutionary pathways are known, a return to a more precise consideration of the effects of selection following speciation can be

undertaken. In particular the classical effects of polyploidy leading to increase in cell size has implications for the fitness and niche of a plant. This talk outlines how selective forces can influence the legacy of polyploidy on simple characteristics within a polyploid series such as *Senecio*.

1.10.1. Ecophysiological aspects of desiccation tolerance in Angiosperms

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A majority of vascular plants are killed by drought if the foliage water potential falls to -15MPa . Others have leaves that survive -300MPa or even $<-600\text{MPa}$.

Even if some desiccation tolerant angiosperms occupy a restricted habitat, they occur over a wide range of climates, latitudes, altitudes, insolation, topography & substrates. DT mechanisms are compatible with almost the full scope of eco. adaptation. Taxonomic distribution of foliar DT ranges broadly across angiosperms, but the tolerance is expressed in only a small proportion of the monocots & even fewer dicots. Aspects of taxonomic spread of DT imply existence of predisposition in certain taxa to evolve desiccation drought tolerant foliage. In some taxa mature & immature leaf tissue are desiccation tolerant; in others only immature leaf tissue is tolerant, being consistent with the view that in the evolution of the phenomenon in angiosperms DT spread from young to mature tissue. Senescence compromises any potential for DT in vascular plants. Interspecific hybrids of "resurrection" species are also desiccation tolerant and mechanisms of DT are phenotypically compatible between species and even genera.

1.10.2. Desiccation tolerance in Bryophytes: the primitive strategy for land plants?

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Green plants are believed to have colonized the land from a fresh water origin requiring adaptive mechanisms that permit avoidance or survival of dehydration. We hypothesize that primitive plants were vegetatively desiccation tolerant and that the primitive tolerance mechanism is reflected in the way modern day bryophytes survive drying. Evidence is consistent with bryophytes employing a strategy of constitutive cellular protection coupled with the induction of a repair mechanism upon rehydration. Proteins synthesized following rehydration (rehydrins) are important components of desiccation tolerance in bryophytes. Genomic studies reveal that some prominent rehydrins are similar to seed LEA proteins, classically ascribed a protective function during drying. The role of LEA proteins during rehydration is unknown but recent data suggests a function in stabilization and reconstitution of membranes. Phylogenetic rehydrin mapping studies led to a re-examination of the evolution of desiccation tolerance in land plants. The new phylogeny suggests that vegetative desiccation tolerance may have evolved from a mechanism first seen in propagules.

1.10.3. Mechanisms of dealing with mechanical stress associated with water-deficit in a range of desiccation-tolerant and -sensitive species

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Vegetative tissues of resurrection plants are desiccation tolerant. Mechanical damage caused by drying is avoided. In desiccation-sensitive plants it is manifest by plasmalemma tearing and wall collapse. We examined tensile properties in leaves of several resurrection and sensitive species during drying in relation to subcellular changes. Tensile strength was greater in wet tissues of tolerant relative to sensitive plants and decreased (dicots) or remained unchanged (monocots) in the latter during drying. In tolerant species, it increased on drying in all species except grasses, where it remained unchanged. When tolerant plants are rapidly dried, so that protection does not accumulate, tensile properties are similar to sensitive species. Mechanical stabilisation in *Craterostigma* spp occurs by wall folding, explaining increased tensile properties when dried. In *Xerophyta* spp walls remain unchanged anatomically and stabilisation occurs by maintaining

cell volume though filling of vacuoles with compatible solutes. In grasses a combination of wall folding and vacuole filling occurs, the counteracting effect enabling unchanged tensile properties.

1.10.4. Programmed cell death in desiccation tolerant and intolerant seeds

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Hardly any animals and higher plants can survive desiccation, but the majority of the latter have desiccation tolerant 'orthodox' seeds. Free radicals, formed during water removal, impair metabolism and necessitate protection and repair during desiccation and rehydration, respectively. Consequently, desiccation tolerance and prolonged longevity in the desiccated state depend on the ability to scavenge free radicals, using antioxidants such as glutathione, ascorbate and tocopherols. The major intracellular antioxidant consistently found in all desiccation tolerant life forms is glutathione, making it essential to survive desiccation. However, if the antioxidant system eventually fails during long-term desiccation, substantial amounts of glutathione are oxidized and its redox potential increases towards more positive values. We present a model of biochemical pathways, suggesting that glutathione redox potential acts as a signal that initiates programmed cell death. Meta-caspase activated DNases finally cleave DNA into inter-nucleosomal fragments, restraining recovery, in both desiccation tolerant and intolerant seeds.

1.10.5. Desiccation tolerance of epiphytic bryophytes in relation to microclimate in a Venezuelan cloud forest

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The forests of the northern Andes are a major centre of epiphytic bryophyte diversity and abundance. We analyse wet and dry-season data for temperature, PAR, r.h., and periods with and without precipitation, in relation to data on desiccation tolerance and light responses of some epiphytic species. Most wet and dry periods lasted no more than a few hours; the longest dry periods recorded in the lower canopy were c. 199 h in February (dry season) and 56 h in August (wet); a quarter of all dry periods were >15 h in February and >8 h in August. Relative humidity was mostly $>70\%$ and often $>90\%$, especially at night. With high r.h. and temperature around 15°C , saturation deficits are small and evaporation slow, so mosses will generally retain turgor over dry periods up to a few hours. All the species examined tolerated at least a few days' desiccation, but all survived and recovered best if desiccated at r.h. $>70\%$; light responses were consistent with measured lower-canopy PAR levels.

1.10.6. Photosynthetic activity of *Haberlea rhodopensis* dehydrated to different extent in natural conditions

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Photosystem II (PSII) function was studied in the leaves of resurrection plants *Haberlea rhodopensis* during desiccation in natural conditions and the subsequent rehydration. Thermoluminescence (TL) measurements showed a clear correlation between the changes in the intensity of B- and A- band in TL glow curves with increasing dehydration. The T_{max} of the two bands however were practically not changed. The observed stabilization of charge storage in PSII was accompanied by a strong reduction of the TL B-band amplitude, showing a reduction of the number of the operating PSII centers starting at the beginning of dehydration. The rate of oxygen evolution and electron-transport activity dropped more slowly and a threshold at 35-40 % RWC was observed. At this state of dehydration the non-photochemical quenching showed maximal values and sharply decreased with increasing water deficiency. After rehydration of the detached leaves in the dark the PSII functioning was quickly restored. Possible mechanisms for the protection of the photosynthetic apparatus in *Haberlea* were discussed.

1.10.7. Landscape patterns of vascular resurrection plants: examples from the Western Ghats of India

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Desiccation tolerance is a rare adaptive trait in vascular plants. Only about 300 species of angiosperms are known to withstand complete desiccation with monocots outnumbering dicots. Desiccation tolerant plants occur preferentially on rock outcrops in the tropics. Contradictory to the expectation of these plants being rare or unimportant, they form dominant vegetation type in some landscapes. Compared with other regions, sparse information is available on desiccation tolerant species in India. Tripogon (Poaceae) is most important amongst Indian resurrection plants with five species, of which three are endemic. An association of poikilohydric Tripogon lisboae dominates vertical cliffs of Northern Western Ghats which are characterized by highly seasonal climate. The potential habitat of cliff dwelling Tripogon sp. was mapped using slope analysis and extent of distribution was estimated. Western Ghats cliffs are the largest known example of landscape level dominance of resurrection plants where they form a dense vegetation cover on steep vertical cliffs.

1.11.1. Identifying new components in the guard cell signalling network

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Guard cells are an excellent model in which to investigate signalling in plants. Their responses to hormonal and environmental signals are easy to measure and provide a highly robust readout against which to test the effects of genetic manipulation of, or pharmacological intervention in, guard cell signalling. A complex signalling network involving changes in guard cell turgor, gene expression, membrane trafficking, cytoskeletal organization and sugar metabolism underlies stomatal movements. The best understood signalling system in guard cells is the closure response stimulated by ABA. Currently we know rather little about how the components involved in ABA signalling are organised in guard cells. Evidence will be presented that guard cell signalling exhibits properties that reminiscent of the emergent properties found in scale free networks (Barabasi and Oltvai 2004, *Nature Reviews Genetics* 5, 101-113) and that increases $[Ca^{2+}]_{cyt}$ might represent a “hub” in this system. In addition recent results concerning the identification of novel components involved in guard cell signalling will be presented.

1.11.2. Guard cell signal transduction from genomics to membrane signaling

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Guard cells have been developed as a model system for dissecting early signal transduction mechanisms. Previous studies have shown that two classes of Ca^{2+} -induced stomatal closing can be separated: rapid Ca^{2+} reactive and long term Ca^{2+} programmed stomatal closing (Allen, et al., *Nature*, 2001 411:1053). However, no genetic evidence exists for Ca^{2+} sensors that function in stomatal movements. Plant genomes include many large gene families producing redundancy which can limit conventional genetic screens. To “narrow down” candidate Ca^{2+} sensor genes for functional characterization we have developed a “single cell-type genomics” approach using guard cells (Leonhardt, Kwak, et al., 2004, *Plant Cell* 16: 596). Using this platform we show important roles for guard cell-expressed Ca^{2+} dependent protein kinases that function in ABA- and Ca^{2+} -activation of anion channels and interestingly also in ABA activation of Ca^{2+} permeable channels. In addition to ABA, high CO_2 concentrations close stomata, but the CO_2 signaling production mechanisms remain less well understood. Data will be presented showing new CO_2 signal transduction mechanisms.

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1.11.3. MJ and ABA signaling cascades in guard cell

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Signalling events during abscisic acid (ABA) or methyl jasmonate (MJ)-induced stomatal closure were examined in Arabidopsis. Stomata of jar1-1 mutant plants are insensitive to MJ, but able to close in response to ABA with a lesser sensitivity to ABA than wild type plants. Reciprocally, the stomata of ost1-2 are insensitive to ABA, but able to close in response to MJ despite to a lesser extent than wild type plants. Both MJ and ABA promote H₂O₂ production in guard cells, while exogenous application DPI, an inhibitor of NAD(P)H oxidases, results in the suppression of ABA/MJ-induced stomatal closure. ABA elevates H₂O₂ production in jar1-1 guard cells but not in ost1-2, whereas MJ induces H₂O₂ production in ost1-2 guard cells, but not in jar1-1. MJ-induced stomatal closing is suppressed in the NADPH oxidase double mutant atrbohD/F and in the outward potassium channel mutant gork1. Analyses of the kinetics of cytosolic pH changes and reactive oxygen species (ROS) production show that the alkalinisation of cytoplasm precedes ROS production during the stomatal response to both ABA and MJ.

1.11.4. Protein phosphorylation and dephosphorylation in blue light response of stomata

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Phosphorylation and dephosphorylation played an important role in blue light response of stomata. Blue light perceived by phototropins, a blue light receptor, is transduced into activation of the plasma membrane H⁺-ATPase via phosphorylation. However, the mechanism by which the signal from phototropins is transmitted to the H⁺-ATPase is unknown. We report here that protein phosphatase 1 functions as a positive regulator in this process in guard cells. We cloned protein phosphatase 1 from *Vicia* guard cells and determined its predominant expression in guard cells. We constructed the mutant protein of phosphatase 1 that lacked activity, and the mutant was transiently expressed in *Vicia* guard cells. Overexpression of the mutant in guard cells specifically inhibited blue light-dependent stomatal opening in the epidermis. The results suggest that protein phosphatase 1 functions as a positive regulator in blue light signaling. We will provide other supporting data for this result.

1.11.5. Nitric oxide is a signaling component during stomatal closure induced by methyl jasmonate or bicarbonate

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Nitric oxide (NO) participates in stomatal responses to abscisic acid (ABA). We report the importance of NO during also methyl jasmonate (MJ) or bicarbonate induced stomatal closure, in epidermal strips of *Pisum sativum* and *Commelina benghalensis*. The presence of SNP (a generator of NO) promoted stomatal closure by MJ or bicarbonate. Suppression of NO levels, by c-PTIO (NO-scavenger) or L-NAME (inhibitor of NO synthase) reversed stomatal closure by ABA or MJ. Real time monitoring with fluorescent DAF-DA indicated that NO production in guard cells peaked at 18 min, compared to 30 min in case of ROS and 15 min in case of pH. The changes in the NO-levels of guard cells and responses to NO-modulators were more pronounced in case of MJ than that of ABA. Our results suggest that changes in guard cell pH and NO are related. However, EGTA reversed stomatal closure, despite the increase in NO-production by MJ or bicarbonate.

1.11.6. ABA signalling in guard cells revisited - New insights from electrophysiological studies in intact plants

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Fast ABA responses - exemplified by half times of stomatal closure around 5-10 min - is believed to not involve gene activation. Instead stomatal closure is accomplished by the release of potassium ions and chloride as well as the metabolic degradation of the major organic anion malate. In search for ABA signalling intermediates the response of ion channels of guard cells in

epidermal peels as well as guard cell protoplasts and vacuoles have been challenged with well-characterized modulators effective in signal transduction pathways of animal cells. Isolated, experimentally well controlled guard cell preparations, however, often lack communication with neighbouring cells, turgor or cytosolic components. Current models, gained from observations on different guard cell preparations, species or not even guard cells are very complex and most likely wrong. To online record changes in ion fluxes across the plasma membrane of guard cells in intact plants, we have developed a method, based on multi-barreled microelectrodes introduced into the cytoplasm of these sensory motor cells. This approach in previous studies was successful in exploring blue- and red light as well CO₂-signalling. Using this online, *in planta* approach together with ABA signalling mutants, we have been able to identify signalling elements required for fast ABA-induced stomatal closure. A model on the ABA-based regulation of guard cell ion transport will be presented at the meeting.

1.12.1. What controls diversity in broad-leaved forests?

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Despite climatic relations at broad scale, biodiversity at finer scales is more variable and sensitive to substrate and biotic factors that are hard to identify and quantify. Diversity in relevés in eastern North America is high at intermediate moisture but erratic in warmer areas; woody and non-woody groups respond differently. Existing hypotheses are examined, especially for niche width and range size. Warming responses by broad-leaved forests might include: - initial increases due to weedy invaders, slower increases with less vagile invaders - increases as forest canopies open, but possible decreases as forest microclimates dry out - gradual decrease as long-living canopy trees die out and are not replaced - decreases as populations of sensitive forbs die out faster than they can recolonize. There is evidence for most of these responses, but will insight into diversity controls predict responses to change? This may require more physiological and other knowledge than we possess.

1.12.2. Global gradients of vascular plant diversity

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For the first time we present analyses of latitudinal gradients of species richness for a group as large as the vascular plants on a global scale. The analyses are based on species richness figures for some 2460 operational geographical units such as countries, islands, mountain ranges, or national parks. The comparison of different species richness gradients in different continents is used to demonstrate the variety of trends and influences mixing up in the overall pattern. One of the most important results is that there is no universal latitudinal gradient of plant diversity. However, in accordance with the water-energy hypothesis, constraints imposed by the physical environment, such as the length of the thermal vegetation period or water availability, show high correlations to large scale trends of biodiversity. These trends are modified by historical influences such as long term climatic stability as well as areas with a high diversity of the abiotic parameters - the geodiversity. Highest species richness values can be found in the Tropical and subtropical moist broadleaf forests followed by tropical and subtropical coniferous forests.

1.12.3. The advantages of being evergreen

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Conspicuous changes in the composition of deciduous broad-leaved forests have been observed in recent years at the southern foot of the Alps (i.e. in southern Switzerland and northern Italy). Since the early 1970s, the number and frequency of evergreen broad-leaved species is steadily increasing suggesting that this particular plant functional type may profit from changing environmental conditions. In this presentation, possible advantages of evergreen versus deciduous broad-leaved species are presented and discussed in the context of climate change and the global exchange of persons and goods. The trend towards milder winter conditions and prolonged growing season in areas with a temperate climate seems to favour evergreens and to increase their competitiveness against deciduous species. A shift from the deciduous broad-leaved to evergreen broad-leaved

vegetation type is presented and exemplified on the species, community and biome level.

1.12.4. Does global change contribute to diversity in broad-leaved forests or is it only a "disturbance"?

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There are two main types of temperate broad-leaved forest, evergreen (warm-temperate to subtropical) and deciduous (typical or cool-temperate, plus some warm-temperate). These are separated by various aspects of winter temperature, as related also to moisture and topography. There is already evidence of evergreen broad-leaved species moving into nearby deciduous forests -- and even more into conifer plantations (that stay warmer in winter). The invading species, however, may be native trees of adjacent forests or escaping planted exotic species. Dispersal mechanisms (e.g. birds) and germination ability suggest which kinds of both smaller and larger species that might be relatively mobile under warming. On the other hand, will the newcomers likely represent a real augmentation of natural diversity, as "new native" species, or will the newcomers mostly be exotics taking advantage of a new kind of disturbance? Some insight may be gained from the so-called Jomon Period in Japan (about 6000 years ago), when southern species moved north and some still remain, as relicts.

1.12.5. Forecasting biodiversity response to global change in broad-leaved forests: a role for plant functional types

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A generic method of vegetation classification using a plant functional grammar and a minimum set of plant functional attributes was applied to a representative series of broadleaved forest sites from high to equatorial latitudes. Analyses reveal predictable changes in suites of plant functional types (PFTs) along defined physical environmental gradients. These studies suggest sieving out of certain PFTs under changing physical environments may have irreversible consequences for biodiversity. Case studies from complex humid tropical forest mosaics in Sumatra and the Western Amazon basin illustrate how PFTs used in association with plant vascular species can enhance the prediction of certain faunal groups. These suggest that (a) a radically new approach to vegetation typology may be required for biodiversity management and (b) biodiversity assessment of forests should include contextual, gradient-based sampling of other vegetation types. It is argued that methods of forest typology that include PFTs rather than relying solely on plant species and structure may provide a more efficient basis for forecasting biodiversity response to global change.

1.12.6. Bioclimatic and historical aspects of biodiversity in the broadleaved forests of mainland Northeast Asia

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An analysis is presented of biodiversity in the world's richest temperate-zone deciduous forests, dominated by broad-leaved species of *Acer*, *Fraxinus*, *Kalopanax*, *Quercus*, *Tilia*, *Ulmus* and by temperate coniferous species of *Pinus* and *Abies*. The vegetation in cold-temperate maritime regions belongs to the *Tilio amurensis*-*Pinetalia koraiensis*, in cool-temperate maritime to the *Aceri pseudosieboldiana*-*Quercetalia mongolicae* (both *Quercetalia mongolicae*), in cold-temperate continental to the *Quercu mongolicae*-*Betuletalia davuricae*, and in cool-temperate continental to the *Lespedeza bicoloris*-*Quercetalia mongolicae* (both *Quercu mongolicae*-*Betuletea davuricae*). The cause of the high biodiversity is a strong drying during the late Pleistocene cooling 18,000 BP. Montane refugia with mesic conditions, in combination with the immigration of drought-tolerant species, created a mixture of ecologically contrasting floristic elements linked to different geographical regions. The occurrence of a seasonal period of moisture deficit in late spring is the most important climatic factor dividing forest classes in temperate mainland Northeast Asia.

1.12.7. Potential habitat suitability changes for 135 tree species in the Eastern United States following climate change

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Evidence mounts for biological shifts underway due to climate change, and these shifts will increase over this century. Our analysis of 135 tree species in the Eastern United States, using two scenarios of climate change and over 30 climate, soil, and landscape predictors, shows the potential for substantial shifts in species ranges. Our statistical model, DISTRIB, has been modified to use three classification and regression tree processes for best model generation, prediction, and understanding. Regression tree analysis (RTA) produces one tree with drivers of distribution clearly elucidated; bagging trees (BT) essentially averages 30 RTA models via subsets of observations to attain better prediction and allows multiple statistics to evaluate the model; and random forests (RF) essentially averages 1000 RTA models with random subsets of observations and predictors to create the best prediction. Results of the 135 species runs shows northward migration of habitat, calculated via a statistic of 'optimum latitude' for a large proportion of the species. The habitat shifts also produce concomitant changes in tree biodiversity across the region.

1.13.1. Harmful algal blooms: from molecular processes to global ocean phenomena

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Algal blooms range from harmless water discolorations, to species that cause mortalities of finfish in aquaculture, and those that produce neurotoxins which can kill human consumers of seafood. Since many blooms are nearly monospecific, correctly assessing the taxonomic and genetic identity of the organisms is crucial in deciding whether knowledge from similar blooms can be applied to the local conditions. Evidence for an alarming global increase in the past three decades in the frequency, intensity and geographic distribution of HABs has been partly attributed to increased public awareness and increased utilisation of coastal waters for aquaculture. However, coastal eutrophication, global climate change and ship ballast water are contributing to the increasingly detrimental effects of algal blooms on fisheries, aquaculture, human health, tourism, marine environment and regional economies. Molecular approaches and remote sensing are now providing us with the forensic tools to resolve the key mechanisms responsible for algal blooms, allowing human society to make well-informed decisions on adequate control, management and mitigation strategies.

1.13.2. Long-term effects of coastal blooms on phytoplankton community structure

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We used a 16-year multidisciplinary database in the Perdido River and Bay system (NE Gulf of Mexico) to determine responses of phytoplankton assemblages and associated coastal food webs to natural and anthropogenic nutrient loading. Orthophosphate and ammonia loading from a pulp mill generated a series of plankton blooms dominated by diatoms, raphidophytes, dinoflagellates and cyanobacteria. Blooms followed species-specific seasonal patterns. Interannual phytoplankton trends were associated with nutrient loading and rainfall cycles. Initial diatom blooms were replaced by raphidophytes and dinoflagellates in time. Bloom effects on phytoplankton assemblages included initial reductions of species richness and diversity, competitive exclusion of various species, direct and indirect replacement processes, and altered nanoplankton trends. Long-term changes indicated complex adaptive processes that led to non-linear responses to nutrient loading and time-based alterations of interspecific relationships. Changes in phytoplankton composition were associated with damaged food webs, population losses and reduced secondary productivity.

1.13.3. Eutrophication trends and consequences in the inshore-offshore system of the Aegean Sea, Eastern Mediterranean

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The Aegean Sea is one of the most oligotrophic areas of the eastern Mediterranean but certain inshore regions within the Aegean Sea were characterised as mesotrophic and eutrophic on the basis of environmental and phytoplanktonic parameters recorded during the period 1996 - 2003. A total of 35 stations were sampled from the northern to the southern open waters and from inshore to offshore waters of the Aegean Sea. Data of nutrients, chlorophyll *a*, and primary production were analysed statistically on the basis of probabilistic statistics in order to classify the trophic status of the examined regions. The levels and limits of the values of each one of these parameters were defined, scaled and summarized by the expression 'open oligotrophic' < 'offshore mesotrophic' < 'inshore eutrophic' waters. Underwater light penetration, and phytoplankton cells (quantitatively and qualitatively) also were recorded. The effect of the trophic status of the water on phytoplankton community structure was evaluated on the basis of the patterns of seasonality, species composition and diversity, and the development of harmful algal blooms.

1.13.4. How do harmful raphidophyte blooms occur predominantly over diatoms in coastal sea?

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Raphidophytes such as *Chattonella* spp. and *Heterosigma akashiwo* have recurrently caused harmful algal blooms accompanying mass mortalities of cultured fish in coastal waters. Diatoms also form red tides with no harmful effects usually. Diatoms generally show faster growth rates than raphidophytes; still raphidophytes cause red tides. How can they? Raphidophytes and diatoms have benthic cysts and resting stage cells in their life cycles, respectively. It is empirically known that raphidophyte blooms have been observed when diatoms are scarce in water columns. Diatoms tend to form resting stage cells under nutrient-deficient conditions, and rapidly sink to bottom and disappear from the water columns. In addition, Raphidophyte cysts can germinate in the dark, whereas diatom resting stage cells require light for germination. Thus the predominance of raphidophytes might be related to the disappearance of diatoms and subsequent failure of germination of their resting stage cells under low light conditions. The selective germination of cysts at the sea bottom is presumably a significant factor for the initiation and success in raphidophyte red tides.

1.13.5. Bacterioplankton community structure as affected by different harmful algal bloom events in W Mediterranean coastal waters

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The CARD-FISH protocol was used to assess the spatial and temporal variation in abundance and composition of dominant groups of bacterioplankton during different harmful dinoflagellate bloom events in the littoral of the W Mediterranean Sea. The composition of the bacterial community differed considerably during the different bloom phases studied. *Cytophaga/Flavobacteria* (CFB) were abundant in the development phase and comprised 47% of total cells assigned to the main bacterial groups (CFB plus γ - and β -proteobacteria). During the maintenance phase, the proportion of CFB decreased, whereas β -Proteobacteria increased. The β -Proteobacteria became the most important group (71%) during the non-bloom period. The β -Proteobacteria maintained low proportions (7-11 %) at all time. During the spatial variability study, β -Proteobacteria dominated the bacterial community (51 %), followed by CFB (35 %), while β -Proteobacteria comprised substantially lower proportions (13 %). This community structure was similar to that observed during the maintenance phase of the bloom in the temporal study, which suggested that this bacterial community

structure is typical for dense phytoplankton blooms in the studied coastal waters.

1.13.6. Potential effects of global climate change (elevated CO₂, temperature and UVB) on phytoplankton population composition

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We are living in an era of rapidly changing climatic conditions with CO₂ set to at least double in the next 100 years, sea surface temperatures to rise by an average of 2-3 °C and with an increase in UVB fluxes at high latitudes. Using data from the literature, we will present physiologically based predictions, substantiated where possible with available data on aquatic systems, about the likely consequences of climate change on phytoplankton composition. For example, microalgae show species specific responses of photosynthesis and growth to elevated CO₂ and UVB - might we then see changes in population structure related to these factors? Since individual species have distinct temperature ranges, even small increases in temperature could have significant effects on population composition, as well as causing earlier onset of spring blooms. Temperature changes can also affect the onset of stratification in the water column- indirectly this can influence community structure through effects on the supply of nutrients from deeper water. All aspects of predicted climate change are thus likely to have major effects on the function of aquatic ecosystems.

1.14.1. Evolutionary systems theory as transdisciplinary methodology. The legacy of Ludwig von Bertalanffy

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Ludwig von Bertalanffy is known as founding father of the General System Theory (GST). When he created his GST amidst the last century, he was able to overcome the deep cleft between the controversial theoretical approaches to biology - mechanism and vitalism. He formulated laws of organisation ruling biota and after generalising them he successfully applied them to different domains such as medicine, psychology, psychotherapy. His main concern was the fate of the human civilisation and the new way of thinking necessary for the survival of mankind. Evolutionary systems theory - as a theory about evolving systems and as a theory that is the result of the merger of systems theory and evolutionary theory - is the most recent elaboration of Bertalanffy's original GST-ideas. It revolves around the notion of self-organisation. It provides a transdisciplinary framework for consilience throughout science.

1.14.2. Scientific and 'Non-Scientific' Ways of thinking

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First various aspects of language and communication are addressed. Then we consider worldviews and scientific theories, which will lead us to four perspectives or ways of thinking: the systematic, systemic, symbolic and symbiotic views, with which we can look at the world. In our Western society, especially the logical/ systematic perspective is common (motto: 'either - or'), which corresponds to classical natural science. Additionally, also the cybernetic/ systemic view (motto: 'as well as') can be found in the social sciences, and the constructivistic/ symbolic view (motto: 'on one hand - on the other hand') refers to the humanities. However, the holistic/ symbiotic view (motto: 'two sides of ONE coin') fits in better with the spiritual realm that plays an important role in traditional societies. Altogether, these considerations encourage a transdisciplinary perception.

1.14.3. Monte Grande, disturbed forest: a semiotic explanation

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The aim of this work is to show how the ecological research and the production of meanings are similar. The knowledge of the world is through the interpretation of the representations and models of the reality, for example, the structural analysis of vegetation to study Monte Grande's cloud forest (Hidalgo, Mexico).

Data of flora, basal area, density, vertical and diametric structure, from the perspective of Semiotics will be representations of the forest. After the interpretation of these elements and their relationship, and after the structural comparison: minimal value of basal area, poor density of tall trees with large BHD, high density of multistem trees, and the increase of the relative value of favored species. These findings indicated that this is a damaged forest. The only possible way to arrived at this conclusion was by the study of representations of the forest and their comparison with representations of other forests. As we can see, the study of a ecological problem and the production of meanings are similar because ecological knowledge, as well as knowledge of the world, is result of the analysis of forest' representations not of the forest itself.

1.14.4. Therapeutic Horticulture in Austria

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Historically gardening and horticulture constituted important components of work therapy programs operating in mental health institutions. The main orientation was food production. Since the late 1970's there was a paradigm shift towards programs providing more positive, value added and reinforcing experiences. In the last years horticultural therapy has been developed as a special domain in public health.

In 2002 the first international congress on therapeutic horticulture took place in Vienna - organised by Austrian Association of Horticulture. It took occasion to celebrate it's 175'th anniversary by supporting the development of therapeutic horticulture.

Research on therapeutic horticulture is still scarce in Austria. There are treatises from various aspects which should be connected in the future: landscape architecture, sociology, botanic and agriculture, occupational therapy, education and psychology, medical and health care. The need for applied research is central in gaining recognition and acceptance.

Since October 2004 a group of experts of the different science and occupations work at the specification of horticultural therapy.

1.14.5. The influence of radiesthetic phenomena on growth, yield and fruit quality of strawberries (*Fragaria ananassa*)

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The effect of radiesthetic phenomena on plants is a less known subject. Plants react to radiesthetic influence with stunting, shifted growth, devitalized constitution and sometimes even with dying. In 2004 a field trial experiment in eastern Austria should clear up this phenomena on growth, yield and susceptibility to pests and diseases of strawberries. In addition fruit quality characters were analysed: P-value (pH-value, conductivity, redox potential), dry matter content, emission of biophotons, fruit-shape-index, mechanical strength of fruit and others. While there were no differences in growth and yield characters, analysis of biophoton-emission showed a higher vitality of fruits on plants with radiation up to a certain level. A relationship between healthy leaves and dry matter content of leaves was also found. Data from P-value and biophoton-emission were comparable. Experiments to check the influence of radiesthetic phenomena on other fruit species are planned.

1.15.1. Biodiversity in organic agriculture (OA)

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OA generates goods benefits through contributing significantly to the conservation and enhancement of agricultural biodiversity. Historically, farmer-designed biodiversity is represented by crops and livestock integrated and linked to a diversified crop rotation that is considered as the key element of OA. The broader spectrum of associated biodiversity including weeds and beneficial plants, soil fauna, herbivores, carnivores and composers is mediated through diversified planned biodiversity. These respond with increased abundance and/or species richness. Biodiversity in OA provides agroecosystem services beyond the production aspects, such as regulation of abundance of undesirable organisms, pests and pathogens, better use of natural resources and agroecosystem stability in general. Better knowledge of the interdependencies of the organisms and habitats involved will lead to more precise agroecosystem management, thus benefiting the biodiversity components supporting sustainability. The associated

biodiversity will thus become a more and more productive and planned biodiversity.

1.15.2. Biodiversity in some organic farms, Thailand

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Biodiversity of three organic farms in Thailand was surveyed in 2004. After harvesting rice, pumpkin (*Cucurbita pepo*), *Sesbania* sp., sword bean (*Canavalia gladiata*) and melon (*Cucumis melo*) were grown in 'Poa Man Samsee' rice organic farm and were tilled into the soil to improve soil fertilizer. The liquid bioextract made from fish, vegetable, and microorganisms was filled into farmland. It increased high yield and small animal in puddle fields. The mixed crop farm of 'Poa Prayong' cultivated rice (*Oryza sativa*) as the main crop. Many kinds of bamboos (*Bambusa membranacea*, *B. nutans*, *Dendrocalamus asper*) were grown as natural guard rows. Mango (*Mangifera indica*), calamus (*Calamus diepenhorstii*) and banana (*Musa sapientum*) were grown around rice puddle. Many kinds of vegetable such as papaya (*Carica papaya*), cow pea (*Vigna unguiculata*), egg plant (*Solanum melongena*), and rose apple (*Syzygium malaccense*) were cultivated in the organic vegetable farm of 'Rai Ton Nuei'. Soil microorganism was mainly sprayed with irrigation. Indian hemp (*Crotalaria juncea*) seeds were sown in bed during vegetable rows for fixing nitrogen and improvements in pest management.

1.15.3. Biological control of apple blue mold with *Pseudomonas fluorescens*

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Pseudomonas fluorescens isolate 1100-6 was evaluated as a potential biocontrol agent for control of apple blue mold caused by *Penicillium expansum* or *P. solitum*. Both the wild-type isolate 1100-6 and a genetically modified derivative, labeled with the gene encoding the green fluorescent protein (GFP) were compared. The *Ps. fluorescens* isolates with or without GFP equally reduced growth of *Penicillium* spp. and produced large zones of inhibition in dual culture plate assays. Cell free metabolites produced by the bacterial antagonists reduced colony area of the *Penicillium* isolates by 17.3 to 78.4%. *Ps. fluorescens* with or without GFP significantly reduced apple decay severity and percent incidence of infected wounds caused by two *P. expansum* isolates after 11 days at 20°C and by *P. expansum* and *P. solitum* after 25 days at 5°C when the biocontrol agents were applied in wounds 24 or 48 hours before challenging with *Penicillium* spp. These results indicate that *Ps. fluorescens* isolate 1100-6 could be an important new biological control for apple blue mold and should be considered for commercialization.

1.15.4. Diversity and importance of arbuscular mycorrhizal fungi (AMF) in organic farming

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AMF are symbionts in most agroecosystems, colonizing the roots of the majority of crop plants. They absorb mineral nutrients by their extended hyphal network from nutrient poor soils and deliver them to their host plant in exchange for carbohydrates. AMF can also enhance the resistance to root pathogens and abiotic stresses, such as drought. Furthermore, AMF play an important role in the formation of stable soil aggregates that prevent soil erosion. Because of these beneficial effects, it is a challenge to develop management strategies beneficial to AMF and applicable for sustainable agricultural systems such as organic farming (OF). Industrial farming practices are apparently detrimental for AMF, as recent studies indicate declining AMF performance with agricultural intensification. However, little is known about the effect of agricultural practices in OF on the diversity of AMF. An overview over the current knowledge and results of our own studies about the effect of agricultural practices in OF on the diversity and root colonisation of AMF will be presented and discussed.

1.15.5. Scenarios for the development of biodiversity in organic farms Uganda

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The current status of the biodiversity - field crops and accompanying biotopes - in Organic Farms in Uganda can be characterised by a high competition between limited farm land and overgrazing. Further more there is a lack of training and extension, which introduces the organic farmers into the significance of biodiversity in both a diversified cropping system and the integration of biotopes into the farming system, as a basic management tool to offer predators and beneficials suitable habitats. The study is therefore discussing scenarios focussing the potential of establishing biodiversity in Organic Farming. The status quo of biodiversity in Organic Farms is described, based on expert interviews of advisers and researchers. In a second step, the perspectives of biodiversity in Organic Farms are described with scenario technique. The key factors are identified by a systems analysis and an impact- and SWOT- analysis is acquired by stakeholders of the Organic Farming product chain. The scenarios show, the endogen potential to drive the biodiversity by farmers and their limiting factors. A policy paper summarises the perspectives to strengthen the biodiversity in Organic Farms under the specific conditions in Uganda.

1.15.6. Biodiversity in organic smallholder farms of Nakuru District, Kenya

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To get basic information on the management status of organic smallholder farms, but also their biodiversity status, a study was carried out in Molo, Lare and Gilgil administrative divisions of Nakuru district, Kenya, from January 2003 to January 2005. A total of 36 farmers, twelve from each study site representative of the socio-economic classes were involved in the study. Besides several analysis on cropping system, nutrient management, organic matter balance and economy of the farms, an investigation on crop diversity, biotopes, their structures and plant species was carried out. The study shows site, management and individual preferences based diversity of biotopes and species. Where as several studies discuss the potential of biodiversity in organic farms in humid zones, concrete investigation of the biodiversity status in organic farms in tropical environments are scarce. Therefore the overall value of this study is to get first data on biodiversity as a basis for the evaluation of the sustainability of organic farming practises in tropical environments.

1.16.1. Plant fibres for composites: promises, problems and strategies for effective utilisation

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Plant fibres are a valuable source of high quality renewable and biodegradable materials. Paper and paper-based products owe their success to wood fibres made by trees. Similarly, non-wood fibres with their combination of high-modulus, high-strength cellulose microfibrils, layered cell walls and low density can compete successfully with many synthetic technical fibres in a wide range of applications, composite materials being particularly important because of their added value. One of the main perceived obstacles to a greater acceptance of plant fibres is their variability. This is the result of intrinsic plant variability, agronomic practices, etc. and also of poorly developed extraction methods. Solution to this problem requires better understanding of the factors which control fibre structure formation, and hence properties, in plants and development of extraction technologies which take more account of the biological design of these fibres, with its advantages and limitations.

1.16.2. Wood-based nanocomposites

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Nanocomposites based on microfibrillated cellulose, MFC, represent a new way of utilizing the inherently high modulus and strength of cellulose. MFC is obtained by disintegration of cellulose in, for instance, animals, wood, fruits, vegetables or algae.

Bacterial cellulose can also be used. A typical lateral dimension of such fibrils is 30 nm whereas the lengths could be several micrometers. The Young's modulus of cellulose is comparable with high-performance fibers such as aramide (Kevlar™). This is due to the highly packed, extended chain conformation of cellulose in its crystalline forms in nature. The model studies performed, predominantly in France, are likely to inspire an expansion of this research area. One interesting possibility is new nanocomposites mimicking the plant cell wall. In order for materials based on microfibrils to make the transition into industrial technology, consideration of cheap disintegration routes from the cell wall is required. In this context, wood pulp and waste products from the food industry in the form of vegetable and fruit pulp (or cattle feed pellets based on those pulps) are of interest as sources of cellulose.

1.16.3. Structure-property relationships in the loblolly pine cell wall: Interaction with synthetic polymers

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While engineered wood composites continue to make great strides in properties and performance, durability of the adhesive bond remains of utmost concern. To address this issue, as well as other performance characteristics of wood-based composites, progress must be made toward understanding the fundamental nature of cell wall polymer-synthetic resin interaction. This presentation builds on earlier reports by applying nanoindentation to loblolly pine specimens varying in age and microfibril angle. Comparing results from this experimental approach and conventional mechanical bending tests, surprisingly consistent trends were observed for both hardness and modulus. Additionally, experiments on adhesive bond lines prepared with phenol-formaldehyde and isocyanate adhesives, revealed little difference in hardness between resin, wood, and interphase. However, dramatic effects were seen in the time-dependent, rheological properties as distance from the bond line increased.

1.16.4. Bio-nanocomposites based on cellulose nanofiber

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A completely new kind of high strength composite was manufactured using microfibrillated cellulose (MFC) derived from kraft pulp. Because of the unique structure of nano-order-scale interconnected fibrils and microfibrils greatly expanded in surface area that characterizes MFC, it was possible to produce composites that exploit the extremely high strength of microfibrils. The Young's modulus and bending strength of composites using phenolic resin as binder achieved values up to 19GPa and 370MPa, respectively, with a density of 1.46g/cm². To evaluate how the degree of fibrillation of pulp fiber affects the mechanical properties of the composites, kraft pulp subjected to various levels of refining and high pressure homogenization treatments was used as raw material with different phenolic resin contents. It was found that fibrillation solely of the surface of the fibers is not effective in improving composite strength, though there is a distinct point in the fibrillation stage at which an abrupt increase in the mechanical properties of composites occurs. The increase was attributed to the complete fibrillation of the bulk of the fibers.

1.16.5. Cellulose Fibrils: Isolation, characterisation and technical application

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Agglomerated cellulose fibrils embedded in a lignin matrix in the cell wall are a predominant reason for the outstanding specific tensile strength of wood. In order to convert these mechanical properties to practical use for polymer composites, fibrils were isolated out of sulphite pulp and characterised by Scanning Electron (SEM) and Transmission Electron Microscopy (TEM). Depending on the disintegration method, the fibrils have diameters in the nanometer and lengths in the micrometer range. Homogeneous, transparent fibril films and polymer composites with two water soluble polymers were prepared. Tensile tests as well as nanoindentation experiments were carried out to determine

the mechanical properties of the composites. The addition of fibrils led to a significant increase of the Young's modulus, the tensile strength, the elongation to rupture and the hardness. Atomic Force Microscopical (AFM) and TEM investigations provide evidence for a homogeneous distribution and network formation of the fibrils within the polymers. The results of this study and the perspectives of producing new bio-based nanomaterials are promising.

1.16.6. Cell-wall Mimicking Cellulose-xylan Nanocomposites

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The wood secondary cell wall exhibits a hierarchical organisation where the distribution and interactions of the constituents on different scales strongly affects the overall performance and particularly mechanical properties. In this study we have prepared cell wall-like, nanostructured materials by assembly of microfibrillar cellulose, produced by *Acetobacter Xylinum*, and xylans extracted from wood and annual plants. Nanocomposites were characterised by several different techniques including atomic force microscopy (AFM), scanning electron microscopy (SEM) and the properties were evaluated by mechanical testing. AFM analysis shows that the materials were composed of a network of randomly oriented microfibrils with xylans in shape of aggregates between and/or covering the cellulose microfibrils. We found a strong effect of the molecular structure of xylan onto mechanical performance of nanocomposites, particularly with regards to effect of moisture. The effect of other hemicelluloses, as well as refinements of the spatial arrangement of the components of the composite, are currently under evaluation

1.16.7. Nanocomposites of cellulose whiskers and biopolymers

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New nanostructured composites, where both the reinforcement as well as the matrix are bio based are interesting materials for medical and packaging applications. Cellulose is the most abundant natural polymer on the earth and combined with natural polymers like starch, cellulose acetate or poly lactide acid will lead to development to new type of biodegradable and environmental friendly bionanocomposites. This new family of nanocomposites is expected to have remarkable improvement of material properties when compared with the matrix polymers or conventional micro- and macro-composite materials. Such improvements can include a higher modulus and strength, a decrease in gas permeability, increased heat distortion temperature, and an increase in the biodegradability rate of biodegradable polymers. The first phase of our work has been to study the separation techniques of micro crystalline cellulose to be able to obtain cellulose whiskers and processing of nanocomposites. The second phase has been to characterize the nanostructures with atomic force microscope (AFM) and transmission electron microscope (TEM) and study the composites thermal and mechanical properties. Our results show that cellulose whiskers can be obtained from microcrystalline cellulose. These cellulose whiskers are 10-20 nm in diameter and the length varies between 300-500 nm. The prepared composites have a nanostructure and shows improved properties compared to pure polymer matrix.

1.17.1. In the future, botanical information will be everywhere

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Google and rough computations suggest that at least 25,000 institutions serve botanical data, a number probably growing rapidly. In the future, botanical information will be everywhere. How will information from this vast store be discovered, evaluated and integrated? One contemporary answer is a divide-and-conquer technology known as Service Oriented Architecture in which "middleware," imposed between databases and users, provides a single kind of data, so one service might provide digitized specimen records, another taxonomic history for the names in those records, etc. Integrating the results is facilitated by a number of international data exchange standards. I'll describe some of these and give two examples from our Electronic Field Guide Project. One is "guided" taxonomic id keys which, at decision points, make calls to remote databases for data to assist the decision, and at terminals, make calls to produce descriptive

species pages. Second is a kind of “data mule” in the form of JPEG2000 image files, copies of which travel to different human and software agents via the internet to obtain, annotate or deliver images from the field, lab, garden or museum.

1.17.2. The metaherbarium - benefits for data providers as well as users

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Collectively, the 6000 herbaria that are distributed around the world provide the most complete view available of the historical and current distribution of the flora of the planet. But, because of the generally opportunistic way each collection has expanded through its history, it is difficult for potential users, without physically visiting a given collection, to fully ascertain the coverage of its holdings from either a temporal, spatial or taxonomic perspective. However, the enormous value of these collections' collective holdings -- what may be called the global MetaHerbarium -- can be realized via digital sharing of specimen information. Use of common Global Biodiversity Information Facility (GBIF)-endorsed standards for data exchange brings visibility to otherwise unknown collections, and allows participating herbaria to receive attribution for sharing data from any of their holdings that respond to user queries of many types, thus validating institutional and societal support.

1.17.3. Automated georeferencing for natural history collections

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Quantitative geospatial references are critical elements of botanical collection data, because they enable us to integrate information within and beyond the biological domain. Of the various classes of information linked to biological specimens, geospatial coordinates used for mapping species distributions are among the most widely demanded by the scientific community and the general public. Geospatial referencing provides the means to link specimen data to the rapidly growing body of spatial environmental data for interdisciplinary research into complex phenomena. For example, access to geospatially referenced data from specimens provides a quantitative basis for biodiversity analyses and predictive niche modeling and for determining sampling densities of various sites. Providing spatial coordinates for collections has proven a significant challenge. Nearly every specimen is associated with a textual representation of the place from whence it came, but few carry with them quantitative geospatial coordinates. We address here advances made in automating the task of geospatial referencing for biological collections.

1.17.4. Integrating geospatial and natural history collections data in a web-accessible database for New Mexico

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Digitizing herbaria specimen information can facilitate collection management processes (e.g., label generation, annotation history tracking, and transaction documentation), make the collection's holding more widely known and easier to access, and provide basic data of utility to science and the general public. Over the past two decades, herbaria and other biological museum collections throughout the world have initiated numerous, largely independent digitization projects that differ in technical complexity, data model, and basic functionality. In this talk, I will discuss our efforts to provide support for digitization, georeferencing and electronic data access to small to medium-sized collections (10 - 200k specimens) in New Mexico. I will feature aspects that allow collections to become involved even with limited technical capabilities and resources and which allow them to participate in larger data accumulation projects at the state, national and international levels.

1.17.5. Virtual herbaria: challenges and goals

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Although long dreamt of, and debated in many forms, at the time of the last IBC, the Virtual Herbarium as a fully realized entity was in

its infancy. In the intervening years a large number of virtual herbarium projects have sprung up. Significant efforts are now being directed at large scale integration so that the dream of a globally integrated Virtual Herbarium is within reach. In this presentation, an overview of some of the various types of virtual herbarium efforts will be given. The challenges and potential payoff of some technologies unique to those efforts, and for massive integration of them, will be examined. The role of a *de facto* global virtual herbarium in other enabling science such as automated keys and earth observing systems will be examined. Finally, the very special place of virtual herbaria among the rapidly expanding plethora global biodiversity data will be discussed.

1.17.6. Rapid digital specimen image and data capture: a web services solution

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The technology required for digital image capture of specimens has become affordable, if not yet commonplace. Digital imaging projects often do nothing more with images than make them available for web display. However, an image can serve the additional purpose as the basis for label data capture. Along with specimen images, label data, particularly georeferenced label data, is a valuable public product for collections. Our ultimate goal is to reduce the total cost of digital collection data capture by significantly reducing human labor required and total project duration. Clicking the shutter on a digital camera initiates a sequence that culminates with the population of label data and a specimen image into a structured collection database. Significant gains can be achieved by developing appropriate protocols and methodologies, then packaging them as web services. We are using open source and commercial solutions, and are developing solutions where necessary that accelerate the herbarium specimen data capture process. Each of these solutions is embedded into web services, providing benefits such as cross-platform interoperability and scalability.

1.17.7. Georeferencing primary biological collections - validation, cleaning and quality control

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The introduction of the World Wide Web opened up the possibility of exchanging primary species data (specimens, observations and survey data) between institutions and for making them available to researchers and others via the Internet. One of the key aspects of making those data useful is the geocoding associated with them - their coded location, for example latitude and longitude. The process of adding georeferencing information is a major source of error and badly georeferenced records can lead to real loss of quality and thus usefulness. Examination of data currently being exchanged via the Global Biodiversity Information Facility Data Portal, shows great variation in the quality of the georeferencing, and that very few institutions have introduced detailed data quality control procedures for validate the data and their georeferencing. Techniques for checking, validating and cleaning the georeferencing or records have, and are being, developed and refined. This paper examines a range of methods - some stand-alone, some via the use of Web Services - and recommends ways that institutions may introduce these into their Data Quality Control procedures.

2.1.1. The bread of polyploidy

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Wheat and yeast both represent contrasting models to study the genetic and epigenetic changes that occur upon polyploidization. The wheat group contains diploid, tetraploid and hexaploid species. Polyploidization occurred ~ 0.5 Mya for tetraploid wheat (genome BBAA) and only ~9,000 Years ago for hexaploid bread wheat (Genome BBAAADD). Bread wheat is a young allopolyploid with a very large genome and relatively limited amount of genomic infrastructures. By contrast, yeast is an ancient allopolyploid with a small and fully sequenced genome and with extensive expression data publicly available. With the recent sequencing of the whole genome of several yeast species, including six *sensu stricto*

Saccharomyces species that can be hybridized to form interspecific hybrids and allopolyploids, yeast has become a paradigm to study the genetic and epigenetic changes that occur during speciation via allopolyploidization. We describe recent studies on the reprogramming of the wheat and yeast genomes as a result of interspecific hybridization and allopolyploidization.

2.1.2. novel phenotypes, genomic rearrangements, and epigenetic changes in resynthesized Brassica polyploids (Brassicaceae)

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Brassica is a fascinating model for plant domestication because of its spectacular variation in vegetative forms. Key questions that emerge: do ancient and recent polyploidy events contribute to morphological variation? Are genome structure and gene expression changes associated with polyploidy directed or random? By synthesizing 50 lines of allopolyploid *Brassica napus* from the same homozygous diploid parents, we are 'replaying the evolutionary tape' to find out if resynthesized polyploids are similar to each other and to natural polyploids. It appears that both chromosomal rearrangements and epigenetic events play a major role in the generation of new phenotypes in polyploids. The results of these studies suggest that polyploidy is an evolutionary mechanism that leads to dynamic changes in phenotype, genotype, and transcriptome at the early stages in polyploid evolution.

2.1.3. Chromatin organisation and methylation in polyploid and hybrid plant species

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We are studying the interaction of genomes in old and new polyploid and hybrid cereals, petunias and bananas. Our work concentrates on repetitive sequences, their distribution and evolution, and their epigenetics as evidenced by chromatin organisation and methylation. Activation of integrated, but dormant pararetroviruses (EPRVs) can be caused by the introduction of wide hybridization and can lead to disease outbreaks through tissue culture or environmental stresses as has been shown for banana. Complex patterns of chromatin methylation result in different inducibility and expression levels of EPRVs in *Petunia* and vary between accessions. Differences in methylation patterns at symmetrical CpG and CpNpG and asymmetrical sites of a widespread repetitive sequence family were observed in diploid and polyploid *Triticeae* genomes. Diploid species of rye and wheat show uniform methylation signals while allopolyploids have more unevenly distributed methylation patterns indicating *de novo* methylation and demethylation mechanisms when genomes are combined in polyploid and hybrid species.

We thank NERC and the EU- framework 5 programme Paradigm for support.

2.1.4. Silencing of duplicated genes in polyploid cotton

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Hybridization followed by genome doubling (allopolyploidy) is a prominent mode of speciation in plants and it can lead to phenotypic novelty. Polyploidy can have considerable effects on gene expression, resulting in gene silencing and adjustments in transcript levels. Molecular techniques were used to screen the expression of over 2000 transcripts and to assay in detail expression levels of 50 genes duplicated by polyploidy, using multiple organs and genotypes of natural and synthetic cotton polyploids. Silencing of one copy was documented for several duplicated gene pairs and silencing of many genes is organ-specific. Some genes show partitioning of expression patterns between duplicates, with one duplicate copy being silenced in some organs and the other copy being silenced in other organs, indicating subfunctionalization. Comparisons of expression patterns in different synthetic polyploid genotypes suggest that silencing of some genes is directed, but it is stochastic for other genes. These results provide a detailed portrayal of gene

expression changes that occur following allopolyploidy and suggest epigenetic causal factors.

2.1.5. Differential expression of homoeologous loci in allotetraploid cotton

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The two genomes of allotetraploid cotton, *Gossypium hirsutum* (AD₁), were derived from an evolutionarily recent merger of two divergent, diploid progenitors closely resembling extant *G. raimondii* (D₅) and *G. arboreum* (A₂). Single-strand conformational polymorphism analyses have demonstrated that many duplicated loci (homoeologs) that resulted from polyploid formation do not contribute equally to the transcriptome. To date, no large-scale survey duplicate gene expression has been conducted. Using a global assembly of 160,000 *Gossypium* ESTs, we identified 6605 pairs of polymorphisms, including both indels and single nucleotide polymorphisms, between the *Gossypium* A and D subgenomes in 2124 pairs of duplicated loci. Short (~25bp) oligonucleotide microarray probe-pairs targeting these polymorphisms were printed on NimbleGen microarrays and 672 loci homoeologous probe pairs were found to diagnostically distinguish both the A and D genomes of *Gossypium*. Preliminary results indicate that a majority of homoeologous loci are not expressed at equal levels and for many duplicate gene pairs, one homoeolog may be completely silenced.

2.1.6. Maintenance of epigenetic states and interaction of epialleles in diploid and tetraploid Arabidopsis

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Natural or experimentally achieved polyploid formation is associated with genomic rearrangements and/or epigenetic changes in gene expression patterns. We investigate the nature of ploidy-related epigenetic modifications and the trans-interaction between genes in different epigenetic states. Several tetraploid *Arabidopsis* lines, derived from a diploid strain expressing a selectable gene, showed transcriptional gene silencing of the marker, forming inactive "epialleles". Once established, the silent state was inherited to tetraploid and diploid progeny, accompanied by hypermethylation at the silent gene. Combining forward and reversed genetic approaches, we investigate which *trans*-acting factors are involved in maintenance of this ploidy-related silencing. The silent epiallele reduced the expression of an active allele if combined in the same tetraploid (but not in a diploid) genome. The suppressive effect is lasting even after genetic separation from the silencing allele, resembling paramutation, and converting a recessive into a dominant trait. Such mechanisms in freshly formed polyploids could contribute to their rapid evolution.

2.1.7. Transcriptome divergence and mechanisms of non-additive gene regulation in Arabidopsis allopolyploids

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Polyploidy has occurred throughout the evolutionary history of all eukaryotes. Reunification of the evolutionarily-divergent genomes in allopolyploids creates regulatory incompatibilities that must be reconciled. Here we show that transcriptome divergence between two *Arabidopsis* species and their new allotetraploids is not purely neutral. The diverged transcriptomes are reconciled by genome-wide genetic dominance and regulatory interactions leading to chromatin-mediated non-additive gene regulation in various biological pathways. Altered regulatory and epigenetic interactions between FRI and FLC mediate flowering time variation in *Arabidopsis* allopolyploids. We propose a general model that suggests genetic dominance as a mechanism for non-additive gene regulation and subfunctionalization of duplicate genes. Furthermore, microRNAs (miRNAs) play a role in post-transcriptional regulation of the duplicate genes, providing a mechanism for rapid phenotypic and developmental changes in new allopolyploids.

2.2.1. Arbuscular mycorrhiza: general introduction and basic concepts

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Arbuscular mycorrhiza (AM) is a widespread mutualistic symbiosis between roots of most land plants and Glomeromycota microbial fungi known to improve plant growth and nutrition and to protect the plant against biotic/abiotic stresses. AM fungi (AMF), obligate biotrophs dated back to 600 Ma, played a key role in land colonization by early plants. AMF are now present in all terrestrial plant systems to impact on their diversity, stability and/or productivity. Biotechnological applications are feasible. Spore morphology-based taxonomy was classically used but molecular tools, based on PCR amplification and rDNA fingerprinting and sequencing are currently applied for diversity/phylogeny analyses. The intraspecific variation of AMF multinucleate spores is being considered with regard to both genetic and functional diversity studies. Recent advances on AMF genomics include: (i) genomic sequencing; (ii) EST sequencing; (iii) expression of genes involved in AMF development; (iv) idem of those involved in nutrient acquisition and metabolism; (v) functional analysis of expressed genes (vi) proteomics and (vii) AM-induced changes in plant gene expression

2.2.2. The role of mycorrhizal fungi in reclamation of degraded ecosystems

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Mining and coal-fired power stations have led to the build-up of fly ash deposits and spoil banks. Succession on these sites is slow due to low abundance and biodiversity of the microbial populations. The research aims to find out the possibilities to accelerate succession on degraded sites and to develop sustainable revegetation by the use of Phyto-Microbial Complexes (PMC's) tolerant to edaphic stress. The concept is based on introduction of suitable plant species, mycorrhizal symbionts and their bacterial associates with the aim to establish vegetation and microbial community of desirable biodiversity. Arbuscular and ectomycorrhizal fungi together with plant growth-promoting rhizobacteria have shown various positive effects on the nutrient uptake, growth and fitness of the majority of plant species tested. Laboratory and field research is building up knowledge on ecophysiology of plant-microbes associations and further research remains essential for the vital use of PMC's on industrial base. Acknowledgement is given to Ministry of Education, Youth and Sports of the Czech Republic for support of the Research Centre Bioindication and Revitalisation.

2.2.3. Isoprenoid metabolism in arbuscular mycorrhizal roots

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Development of arbuscular mycorrhizal (AM) roots is correlated with accumulation of various isoprenoids, i.e. C13 cyclohexenone derivatives and acyclic 'mycorradicin', the latter being a major component of the long-known 'yellow pigment'. Marked increase of transcript levels of the two pivotal enzymes of the corresponding methylerythritol phosphate (MEP) pathway, 1-deoxy-D-xylulose 5-phosphate (DXS) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), is induced by the fungal symbiont, e.g. *Glomus intraradices*. Two distinct classes of DXS-like cDNAs have been identified. DXS1 genes are preferentially expressed in many developing plant tissues except roots, whereas expression of DXS2 genes is strongly stimulate in roots upon colonization by mycorrhizal fungi. This is accompanied by a dramatic increase of root plastids and the formation of dense plastid networks covering the arbuscules. DXR has been immunolocalized in plastids around the arbuscules, tightly correlated with arbuscule development. These observations point to an important role of root plastids and (apo)carotenoids in the functioning of AM symbiosis.

2.2.4. Role of arbuscular mycorrhizal fungi in phytoremediation - practical and theoretical aspects

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Diverse human activities resulted in the creation of large areas contaminated by heavy metals. Bioremediation is an alternative to

traditional technologies. Considerable attention was focused on the potential use of plant rhizosphere to remediate contaminated soil, however, the role of mycorrhizal fungi, which are an important component of soil microbiota has been almost neglected. Properly developed mycorrhizal symbiosis may enhance the survival of plants in polluted areas by improving nutrient acquisition, water relations, pathogenic resistance, phytohormone production, etc., thus supporting the success of bioremediation. In addition, mycorrhizal fungi were found to play an important role in heavy metal detoxification and vegetation establishment on strongly polluted soils. The effectivity of the bioremediation techniques depends on the appropriate selection of both partners. The experiments carried out on industrial wastes in Poland pointed out that plants conventionally introduced in such places disappear relatively soon, while those appearing during natural succession are better adapted for harsh conditions.

2.2.5. Arbuscular mycorrhizal fungi and heavy metal and salt stress

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Colonization by arbuscular mycorrhizal fungi makes plants more resistant to stresses. In heavy metal stress, an isolate of the AMF *Glomus intraradices* from the zinc violet effectively transfers heavy metal tolerance to plants in heavy metal soils. Inside the roots, heavy metals are concentrated in the inner parenchyma cells with most of the fungal structures. Northern analyses, Real-time PCR experiments and in situ hybridizations showed that plant genes are differentially expressed upon heavy metal stress and mycorrhizal colonization. In particular, the transcript level of a root specific metallothionein is drastically upregulated upon heavy metal stress but downregulated upon AMF colonization. A SSH library constructed from fungal hyphae grown either in high or low concentrations of zinc did not provide any fungal counterpart of plant genes. In contrast, genes of enzymes which detoxify reactive oxygen species were found. Thus the fungal cells in the symbiosis may primarily have to cope with the heavy metal-induced oxidative stress. Plants from salt marshes are also strongly colonized by AMF. Results on AMF and salt will also be presented.

2.2.6. The arbuscular mycorrhizal and the rhizobial symbiosis: similarities and differences

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Legumes can form a symbiosis not only with nodule-forming rhizobial bacteria, but also with arbuscular mycorrhizal fungi (AMF). Abundant information is available on the regulation of the legume-rhizobium interaction, whereas in the understand of the regulation of the arbuscular mycorrhizal symbiosis several steps still remain unclear. Ample evidence indicates that the rhizobial host can control the extent of nodulation, the so-called autoregulation of nodulation. An autoregulatory mechanism has also been postulated for the AM symbiosis. Moreover, an established mycorrhizal association can systemically suppress not only further mycorrhization but also nodulation and the application of Nod factor to one side of a split-root system suppresses mycorrhization on the other side. These results point towards certain similarities in the autoregulatory mechanisms of the two associations. Recent results strengthen this hypothesis, showing that in supernodulating soybean mutants where nodulation is not autoregulated, also mycorrhization is not autoregulated. The regulation in the rhizobial and the arbuscular mycorrhizal association is discussed.

2.2.7. Mechanisms and interactions in mycorrhizal phosphate transport

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The arbuscular mycorrhiza represents an ancient symbiosis, thought to have originated at least 460 million years ago, in the roots of plants pioneering the colonization of terrestrial habitats. In these associations, a key process is the transfer of phosphorus as inorganic phosphate to the host plant across the fungus-plant interface in exchange for plant-based carbohydrates. Phosphate transport across the cell boundary is mediated by transport proteins. The first mycorrhiza-specific phosphate transporter gene, named *StPT3*, was recently cloned from potato (Rausch et al.,

2001). Using molecular-genetic tools, the regulatory mechanism involved in *StPT3* expression was subsequently shown to be conserved in phylogenetically distant plant species, and to be selectively induced by fungal species from the phylum *Glomeromycota* (Karandashov et al., 2004; Schüssler et al., 2001). Novel tools to unravel the molecular interactions involved in symbiotic phosphate transport are presently being established in the group. Recent progress in understanding the regulation and functional role of mycorrhiza-specific phosphate transporters will be presented.

2.3.1. The mesozoic context for the origin of angiosperm plant-insect associations and floral syndromes

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The presence of insect associations on various crown groups of Mesozoic seed plants increasingly place Early Cretaceous flowering plants in a dynamic and comprehensive ecological context. These plant-insect associations extend back to the Middle Triassic, 100 m.y. before the earliest angiosperm fossils, and indicate that host-specific leaf miners, gallers, wood borers, and seed predators were efficiently partitioning foliar, stem, and reproductive tissues of seed plants in both Gondwana and Laurasia. Some endophytic associations likely induced both accommodationist and highly defended strategies to ward off herbivory. Major Mesozoic seed-plant groups, including Ginkgoopsida, Bennettitales, Voltziaceae, Cheirolepidaceae, Pentoxylaceae and Corystospermaceae harbored diverse feeding guilds that set the stage for herbivore colonization of early angiosperms, perhaps by lateral transfer. Additionally, gymnospermous pollination is documented by fossil data from insect gut contents, mouthpart structure, plant fructification damage and reproductive biology, and temporally overlaps with early angiosperm pollination styles.

2.3.2. Multiple meanings and modes: on the many ways to be a “generalist” flower

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Describing a species of plant as being a pollination “generalist” or “specialist” is not straightforward. There are a number of different ways of defining “generalized” and “specialized” and some important conceptual issues in relation to the ecological quantification and evolutionary meaning of generalization. Apparently generalized species may be cryptically specialized (pollinated by only a few species, despite a large diversity of flower visitors), or pollinated by a single functional group of related taxa. In addition, plants that possess phenotypically specialized flowers may in fact be ecological generalists in their interactions with pollinators. Finally, sampling effort in the field is an important consideration, both within a population and across populations. In this paper I illustrate these issues and concepts using case studies from my own work on genera such as *Ceropegia*, *Xysmalobium* and *Lotus*. A more detailed, critical appreciation of generalized plant-pollinator interactions is necessary to our understanding of the ecology and conservation of this important ecological process.

2.3.3. The problem of generalized flowers - morphological aspects

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Certain groups of flowers characterized by similar physiognomy are preferably visited by a diverse group of animals. These flowers have passed a process of mutual specialization to their pollinators. The question for the initial forms of this evolution remains unanswered, but a broad spectrum of visitors is assumed. We prefer to designate them as ‘generalized’ instead of ‘non-specialized’, since each organism needs to be adapted to its environment in any way. Generalized flowers even occur in otherwise highly evolved families. They may have developed secondarily from specialized types combining plesiomorphic with secondarily achieved characters. Thus ‘generalized’ & ‘specialized’ are relative terms. Asking for the generalized flower does not imply the question for the early primitive flower! Neither in fossil records nor among the extant primitive Angiosperms we find a model organism for a flower with a broad spectrum of visitors, displaying all the organs in their plesiomorphic state. The Winteraceae, however, present members coming close to this

ideal with primitive features and open generalized flowers, visited by a broad spectrum of pollen-vectors.

2.3.4. The top-10 most generalized flowering plants in the world

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The ‘pollination generalization level’ L_n of a flowering plant species n , here defined as number of flower-visiting animal species attracted to the flowers of n in a given study site, varies enormously among plant species. I have compiled information about L from the literature and my own studies for more than 1800 plant species throughout the world. The frequency distribution of L is very skewed. In about half of all species $L = 1$, whereas a few species are visited by an extremely diverse fauna. Within study site, L increases with number of flower-visitor species A . In order to correct for this, I express L relatively, as the proportion of the total flower-visitor fauna in a study site that visits a given plant species (L/A). In my talk, I list the top-10 most generalized (L/A) species in the “world”, i.e. out of a sample of +1800 plant species. I discuss why they are on the list: Is it because of flower morphology, abundance, habitat, study methodology etc.?

2.3.5. Generalist flowers and phytophagous beetles in two tropical canopy trees: resources for multitudes

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Using the STRI Fort Sherman canopy crane, Panama, we studied beetle visitors to flowers of *Tapirira guianensis* (Anacardiaceae) (Tp) and *Nectandra purpurea* (Lauraceae) (Np) over 2 flowering seasons. In these unrelated short (Np) and tall (Tp) canopy trees, the superficial morphology of the flowers and inflorescences are similar. Flowers are five-parted, minute (to 2 mm), cream to slightly purplish (Np) and last 1 to 2 days. Inflorescences bloom for about 3 days in Tp and 1-2 weeks in Np. The white floral sprays (inflorescences) in both plants are emergent above the parent tree leaf layer and are visited by numerous beetles. Total number of phytophagous beetles collected from inflorescences was 122 species (841 individuals) and 122 species (723 individuals) for Tp and Np, respectively. Beetles were sorted into 3 functional groups: general flower visitor, species that develop in buds and seed predators. Of the general flower visitors, 96 species and 99 species were collected from Tp and Np, respectively. There were 66 species of beetles in common between the two trees. Such numbers clearly imply that in the tropics generalist flowers are important resources for beetles.

2.3.6. Generalized versus specialized pollination modes in tropical floras: the case of the cerrado vegetation in Brazil

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The prevailing view of plant-pollinator interactions assumes that most tropical communities exhibit higher specificity than in temperate zones. All angiosperms in a one hectare plot of cerrado vegetation in São Paulo State, were investigated with respect to pollination mode and pollinators. The majority of the 301 species were either bee pollinated (38%) or generalists (37%); in addition, 13% were anemophiles, 3% cantharophiles, 2% each ornithophiles, sphingophiles and phalenophiles, and 1% each chiropterophiles, psychophiles and myiophiles. A relative measure of importance of an agent with respect to a particular plant species was obtained by considering the visiting animals as exclusive, principal or additional pollinators. Of the 261 zoophilous species, 51 are “specialized”, that is, are exclusively pollinated by a single animal group. The great majority (210) are effectively pollinated by at least two groups, which act either as principal or additional pollinators. Flowers of these oligo- to polyphilic plants have a relatively low level of floral specialization. Detailed studies of other tropical vegetations might well reveal a similar pattern.

2.3.7. A floral ecologist’s past fifty years: Some personal thoughts and experiences

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After a first flourishing in the 19th century, floral ecology fell into relative dormancy for almost 50 years, although some important innovations were achieved during that time. In the mid-20th century, the field became revitalized, mainly through interdisciplinary impulses: insect and vertebrate biology, physiology, genetics, floral morphology, new interest in tropical floras, and modern methods. Floral ecology is now so expanded and divergent in topics that no single scholar can maintain a full overview. Two different motivations of research prevail: the Post-Darwinian approach involving the evolutionary perspective, guided by causative and reductionist reasoning, and the comparative functional interpretation of floral structure, maintaining the narrative culture of documentation and explanation of existent conditions. In the present contribution, the author outlines his own involvement in pollination science and recalls the circumstances that led him to his findings concerning adaptive radiation into floral syndromes, the actual role of bat pollination, novel types of floral reward, and deceptive attraction.

2.4.1. Reflections on the ultrastructure of softwood fibres

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The ultrastructure and interaction of the wood polymers in the fibre wall of softwood tracheids is of high interest for understanding the mechanical properties of wood fibres. The cell wall structure in terms of cellulose aggregates and the distribution of the hemicelluloses, glucomannan and xylan, will be discussed based on measurements using Atomic Force Microscopy as well as Dynamic FTIR spectroscopy. The cellulose aggregates are shown to have a large variation within the cell wall, however uniform across the cell wall. When processed or subjected to different environments large aggregate size changes are shown to occur. For softwood fibres FTIR showed that of the two hemicelluloses, glucomannan was closely connected to the cellulose while the xylan seemed to be more freely arranged. Taking into account the swelling behaviour of the cellulose aggregates it was likely to assume that the glucomannans are making up some of the structure of the cellulose aggregates being strongly bonded to these. Based on these studies a model for the ultrastructure of the cell wall and how it may change by processing is discussed.

2.4.2. Characterization of the gelatinous layer (G-layer) as origins of the xylem properties peculiar to the tension wood

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Origins of the mechanical properties peculiar to the tension wood (TW) in the arboreal dicotyledon were clarified in relation to the gelatinous layer (G-layer) formation. [Materials] Tested species were (1) *Quercus sp.*, and *Zelkova sp.*, forming a very thick G-layer, (2) *Acer spp.*, forming a thin but clear G-layer, and (3) *Magnolia spp.* forming no G-layer. Eight to ten points were set peripherally in a leaning stem of each tested tree, and the growth stress (GS), the drying shrinkage (SH), and the Young's modulus (MOE), along the fiber direction, were measured. Afterward, area ratio of the G-layer was determined at each point. In *Magnolia spp.*, cellulose content and average microfibril angle were measured. [Results] Intensities of GS, SH, and MOE became larger with increase of the area ratio of the G-layer, which suggests that origins of the TW properties were attributed to the nature of the G-layer. In *Magnolia sp.*, large cellulose content and small microfibril angle produced the similar effect as forming the G-layer. Intrinsic properties of the G-layer were estimated quantitatively by applying the observed results to the simulation using a multi-layered fiber model.

2.4.3. Probing cell wall characteristics of mild compression wood

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Compression wood forms in gymnosperm trees in response to gravity mediated stimulus. The form of compression wood varies greatly, ranging from very mild to severe. This review focuses on cell wall characteristics of mild compression wood, which also occurs in a wide range of forms. Recent investigations employing a combination of various microscopy techniques have provided information that forms an important basis for distinguishing various forms of mild compression wood and also mild compression wood

from normal wood. The single most distinguishing feature, which can be used to differentiate the mildest forms of compression wood from normal wood, is the greater lignification of the outer S₂. However, in some cases the outer S₂ is only marginally more lignified than the corresponding cell wall region of normal wood, and the experience has shown that distinguishing such mild forms requires the use of high resolution microscopy, such as transmission electron microscopy. This and other features of mild compression wood will be described with suitable illustrations during presentation.

2.4.4. The effect of latitude and impact of fertilisation on tracheid dimensions and wood density of Norway spruce (*Picea abies* (L.) Karsten)

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Tracheid diameter, cell wall thickness and wood density were studied in two long-term nitrogen fertilisation experiments of Norway spruce. The purpose was to compare a northern growth site (66°51'N, 27°07'E) to a southern growth site (61°10'N, 26°03'E), with a respect to a fertilisation treatment. Solid nitrogen fertiliser was given every five years during years 1961-2000 and led to 992 and 1306 kg of total N deposition per ha in both sites. Five 60-year-old unfertilised control trees and five fertilised trees were selected in year 2002 from both sites. The fertilisation increased annual ring width significantly in the northern location, but similar effect was not seen in the southern site. Transverse wood sections (thickness 15 µm) were cut with a cryo-microtome at 10 year intervals for cell size measurements. According to our preliminary results, nitrogen fertilisation decreased tracheid size in both sites. The impact of the interaction of northern vs. southern growth site and fertilisation on tracheid properties with respect to wood density will be discussed.

2.4.5. Cell wall formation and polymer topochemical assembly in the developing xylem

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Secondary wall organization is in large part responsible for the mechanical and physiological properties of wood. Using high resolution of transmission electron microscopy (TEM) together with the specificity of immunological probes, we investigated the deposition *in muro* of hemicelluloses and lignins from the early stage of differentiation to the mature fibre and vessel walls in growing plants of *Arabidopsis thaliana* and poplar. TEM examination of differentiating cells as well as various wood and wood-derived materials and genetic mutants brought multiple evidence of the lamellar organization of the secondary walls. Immunogold labelling suggested that two different types of lignin molecules differentially polymerised at different steps of the wall formation, and two structurally different xylan types, had specific functions in the cohesion of cellulose microfibrils (CMFs). This process may be modified in response to environmental factors. A model of the spatio-temporal relationships between hemicelluloses, lignins and CMFs during secondary wall assembly is proposed.

2.4.6. Immunolocalization of enzymes involved in synthesis of syringyl lignin

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Immunocytochemistry is a powerful tool to reveal the localization of enzymes at cellular and subcellular level. Lignification is thought to occur in three steps. Monolignols are synthesized in the cell, transported towards the cell wall, and polymerized dehydrogenatively within the cell wall. Many enzymes are involved in the synthesis of monolignols. We investigated the enzymes involved in syringyl lignin. Immunolabeling of coniferyl aldehyde 5-hydroxylase catalyzing the conversion of coniferyl aldehyde to 5-hydroxy coniferyl aldehyde is mainly observed in the fibers of aspen during the secondary wall formation. It is localized mainly on rough endoplasmic reticulum. Sinapyl alcohol dehydrogenase catalyzing the conversion of sinapyl aldehyde to sinapyl alcohol is also localized in the fibers during secondary wall formation. It is localized mainly on the cyto-sol. Taken together, accumulation of syringyl lignin in aspen is discussed.

2.4.7. The process of xylem cell differentiation in relation to wood properties.

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The physical properties of wood depend on the cell types present, their relative proportions, their distribution (anatomical arrangement), their morphology and structure, and the structure and composition of their cell walls. Whilst these parameters are under genetic control, they are also sensitive to environmental variation. Key factors during development of xylem cells which affect the quality of wood formed are the degree and direction of cell enlargement, the orientation of deposition of cellulose microfibrils in the secondary wall of fibres and the amount of lignification which takes place. This presentation will describe some of the microscopic structural changes which take place during differentiation of fibres, tracheids and vessel elements. In particular, we examine the role of plasmodesmata and the cytoskeleton in cell commitment, enlargement and wall formation. We also present initial results on the differential expression of genes, in particular tubulin genes, in cambium tissues during the formation of juvenile and mature wood.

2.5.1. Genome Size Matters

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Genome size is a key biodiversity character which amazingly varies more than 1000-fold between plant taxa. Interest in plant genome size evolution and its significance has increased greatly with a recent upsurge in the number of size estimates published, including the first values based on 'complete' genome sequencing. Comparative genomics based on robust phylogenies have provided exciting new insights into the molecular mechanisms responsible for genome size gain (obesity) and loss (down-sizing), and have provoked new ideas about the evolutionary forces driving such changes. Knowledge of species DNA amounts has many important uses, given its nucleotypic effects, and recent studies provide the first telling evidence that genome size can be a key predictor of species extinction, and survival in polluted environments. Linking widely diverse perspectives on genome size variation at levels from DNA sequences to ecosystems will be an important driver for progress in many botanical fields.

2.5.2. Mechanisms of plant genome size evolution: insights from comparative genome analysis

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Developments in the analysis of plant genomes over the past 5-10 years have begun to provide much clearer insights into the processes involved in changes in genome size. The variation within groups such as the angiosperms is very large, with an approx. 1000-fold variation, and it is likely that several mechanisms are involved. Genetic mapping of many species and sequencing of a few has revealed that duplication of whole or parts of the genome is probably ubiquitous. This is a powerful method for genome size increase, polyploidy doubles C-value whereas segmental duplications will have smaller effects. Polyploidy and hybridization may also be accompanied by deletion or proliferation of DNA sequences that may also contribute to C-value variation. Transposable elements, particularly retrotransposons, are also key elements in genome size variation, though they do not necessarily proliferate unchecked as recent studies in *Arabidopsis* have shown. The dynamic nature of genome size variation has been highlighted by the application of phylogenetic methods which that both increase and decrease in size occurs within phylogenetic lineages.

2.5.3. Genome obesity and downsizing across the Tree of Life

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Polyploidy and transposon amplification are widely accepted as the predominant mechanisms for generating genome obesity in angiosperms. These observations, coupled with proposals that most genomes are palaeopolyploid, suggest that large genomes should be the rule rather than the exception. However, superimposing genome size data onto a well supported phylogeny

shows that species with large genomes are exceptional and restricted phylogenetically. Further, data showing that C-values in some polyploids are lower than the expected sum of their parental genomes, and molecular evidence showing elimination of DNA in some polyploids suggest that polyploidization may also triggers genome downsizing. Analysis of C-value data across a broad angiosperm sample confirmed that genome downsizing following polyploidy may indeed be a widespread phenomenon of considerable biological significance. Extending these approaches to other plant groups (e.g. gymnosperms, ferns, mosses and algae), although often limited by scarcity of data, further highlights the rarity of large genomes, and suggest that genome downsizing operates over large phylogenetic distances.

2.5.4. Nuclear DNA content estimates in green algal lineages: Chlorophyta and Streptophyta

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An ancient divergence separates eukaryotic green algae into two major monophyletic lineages: Chlorophyta and Streptophyta, and a residuum of green algae, including *Mesostigma* (Prasinophytes). The present investigation expands our database of C-values to 135 species, emphasizing Streptophyta and the charophycean lineage which is sister group to land plants. The DNA-localizing fluorochrome DAPI (4', 6-diamidino-2-phenylindole) and RBC (chicken erythrocytes) standard were used to estimate 2C values with static microspectrophotometry. In Chlorophytes, 2C DNA estimates range from 0.2 - 6.1 pg. For Streptophytes, 2C nuclear DNA contents range from 0.2 - 2.1 pg, excluding the highly polyploid Desmidiaceae and Charales, which have genome sizes of up to 20.7 and 39.2 pg, respectively. These data will help pre-screen candidate species for the on-going construction of Bacterial Artificial Chromosome (BAC) nuclear genome libraries for the Ancestral Green Flagellate (AGF). It is proposed that the ancestral genome for both the Chlorophyta and the Streptophyta lineages is small (c. 0.05 - 0.1 pg).

2.5.5. Genome size - pollution and survival

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In eukaryotes, nuclear genome sizes vary by more than five orders of magnitude. This variation is not related to organismal complexity, and its origin and biological significance are still disputed. We assessed the adaptive value of genome size with a comparative study of grassland plant communities occurring on a gradient of environmental conditions caused by pollution from a former lead smelter. The concentration of Pb in the soil on the five investigated plots ranged from 0.01% on the unpolluted reference plot to 3% on the most polluted plot close to the smelter stack. The number of vascular plant species correlated negatively with the level of metal contamination and ranged from 106 to 20. Analysis of genome sizes of 70 herbaceous dicot perennial species occurring on the investigated plots revealed a negative correlation between the concentration of contaminating metals in the soil and the proportion of species with large genomes in plant communities. These results represent the first direct evidence, based on a field study, that genome size is associated with differential survival of species in extreme environmental conditions.

2.5.6. Does insular environment affect genome size? An evidence from Macaronesian flora.

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Genome sizes for 239 endemic Macaronesian species from 111 genera and 39 families were estimated using propidium iodide flow cytometry. Surprisingly narrow variation in 1C-values was revealed, ranging from 0.19 pg to 9.52 pg (ca. 50-fold difference, thus less than several plant families). Majority of endemic species (67.8%) possessed very small genomes (1C = 1.40 pg), while small (1C = 1.41-3.50 pg) and intermediate (1C = 3.51-14.0 pg) genomes were much less frequent (20.5% and 11.7%, respectively). These proportions dramatically differ from the world flora figures. Moreover, minimum values hitherto known for major phylogenetic lineages have often been approached or even

passed in Macaronesia. Similarly, for nearly all genera and families, endemic members had significantly lower C-values than their non-Macaronesian counterparts. Marked prevalence of very small genomes among unrelated angiosperms indicates that genome miniaturization might be a common evolutionary strategy in insular conditions. Relationship between rapid burst of speciation and genome size is also suggested.

2.5.7. The large genome constraint hypothesis: evolution, ecology, and phenotype

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If large genomes are truly saturated with unnecessary “junk” DNA, it would seem natural that there would be costs associated with accumulation and replication of this excess DNA. Here we examine the available evidence to support this hypothesis, which we term the “large genome constraint”. We examine the hypothesis at three scales: evolution, ecology, and the plant phenotype. In evolution, we found that genera with large genomes are less likely to be highly speciose - which suggests a large genome constraint on speciation. In ecology, we found that species with large genomes are underrepresented in extreme environments - which suggests a large genome constraint on the distribution and abundance of species. Ultimately, if these ecological and evolutionary constraints are real, the genome size effect must be expressed in the phenotype. Therefore, we review data on the physiological correlates of genome size. Most notably, we found that species with large genomes have reduced maximum photosynthetic rates. These correlations may help explain why species with large genomes are trimmed from the evolutionary tree and have restricted ecological distributions.

2.6.1. Phytogeography and epiontology of floras

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Patterns of the distribution of the plants over the earth can be demonstrated to be non-random. The Phytogeography has evolved in order to explain the non-randomicity of plant distribution in terms of the historical and ecological processes. Since of the origin of this scientific field, the central question was the definition of the flora concept, as a means for studying the taxa (or the whole of local populations) inhabiting a given territory, and the delimitation of the spatial scale in which the floras originated and evolved as natural phenomena. The presentations discuss different qualitative and quantitative methods applied for evaluating the floristic diversity and representativeness, for defining the phytogeographical division at regional level and for laying out hypotheses of the flora genesis (epiontology). The open question is: is it possible to find principle and well shared approaches for phytogeographical analysis of floras?

2.6.2. Defining and delimiting choronomic units: methods and problems in the phytogeography of Calabria (S Italy).

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The Calabria region is the southernmost part of Italian peninsula, extending ca. 350 km north to south from 39° 54' to 38° 42' N in the centre of Mediterranean sea; the area is widely mountainous raising to about 2000 m a.s.l. many times throughout its latitudinal range. Often included in the Mediterranean region by a phytogeographical point of view, instead recent works assigned the upper part of mountains to the European region. Our research started from distribution of endemic and relictual taxa for definition of choronomic units and geographic elements, while auto- and synecological data were used as comparative to test their consistence and define their boundaries. Moreover, the distribution analysis of relictual and vicariant taxa, compared to paleogeographical and palynological data, was used to find possible historical pathways that outlined the present choronomic units.

2.6.3. Biogeography of plants of the Guiana Shield: Inferences from seven florulas from Northern South America

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The florulas of seven well-collected locations in northern South America were tested for floristic similarity. Checklists from Kaieteur Falls National Park, Mabura Hill, and Iwokrama (Guyana), Reserva Ducke (Brazil), Saül (Central French Guiana) and Guaramacal and Ávila National Parks (Venezuela) were first synonymized and then the relationship between Sorenson's dissimilarity and geographic distance between florulas was examined using the Mantel Test at the species and genus level. The distribution of species over the sites was influenced by the distances between sites. However, there was no evidence that the distribution of genera between sites was influenced by the distances between sites. The results support a model of floristic similarities determined by the distribution of white sand and sandstone substrates in the three florulas from Guyana while the flora of Saül, although located on the Guiana Shield, lacks white sand substrates and shows more generalized similarities to the other florulas from Northern South America.

2.6.4. Phytogeographic regions of NW Russia, with connection of distribution of the apomictic *Hieracium* species

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The system of phytogeographic regions of NW Russia (Saint-Petersburg, Pskov and Novgorod Regions) is based on the characters of species distribution, landscape and climate. The territory is situated in Southern Boreal and Hemiboreal zones separated by the northern limit of oak and some species of its suite. It belongs to the oceanic sector O1 with three subsectors within (a detailed version of the “Finnish” phytogeographic system). 21 regions (with subregions) are separated on the basis of presence of characteristic (mainly forest, especially thermophilous) species and landscape peculiarities. These regions may be grouped in large-scale traditional provinces (Fennoscandia, Baltia, Belorussia, Middle Russia) with intermediate areas between them. The most diverse apomictic genus *Hieracium* was taxonomically treated anew and the microspecies distribution according to the phytogeographic regions was analysed. The distribution of 55 native *Hieracium* species generally follow the main trends in flora, dividing the territory into 5 “*Hieracium* regions”: Maritime East Fennoscandia, Continental East Fennoscandia, Baltia, North Russia, Central Russia.

2.6.5. The origin and evolution of the Socotran flora - evidence from molecular data

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This study aims at the analysis of biogeographic patterns among the flora of the Indian Ocean archipelago of Socotra. Hitherto, Socotran endemics were purported to have biogeographic relationships to Africa, Arabia, Asia and/or the Canary Islands. The origin of the Socotran flora and the biogeographic history of the Eritreo-Arabian subregion is here reconstructed using molecular phylogenies of seven dicotylous genera with taxa endemic to Socotra. Invasion events from E Africa and/or Arabia are indicated for *Thamnosma* (Rutaceae), *Echidnopsis* (Apocynaceae), *Campylanthus* (Plantaginaceae), *Kleinia* (Asteraceae) and *Aerva* (Amaranthaceae). So far, close relationships to the Somalo-Ethiopian and South Arabian floristic provinces are obvious, and direct connections to the Canary Islands or Asia can be ruled out. Vicariant patterns possibly caused by the geological separation of Socotra from Arabia 35-15 mya could not be detected by creating general area cladograms.

2.6.6. Qinghai-Tibet Plateau Uplift and its impact on Tethys Flora

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Paleobotanical data had showed that the beginning flora of Qinghai-Tibet Plateau was a part of Tethyan Tertiary “paleotropical flora”. After late Paleogene, along with climate of the Mediterranean gradually become aridity, the “laurophyll” or “paleotropical geoflora” gradually replaced by temperate sclerophyllous forest, semiarid or arid the modern Mediterranean flora. In Neogene, Himalayas uplifted caused the semiarid or arid modern Mediterranean flora turning to adapt to different alpine environments. Through analysis of the origin of sclerophyllous

oaks forest in these region and Eurasian and North temperate disjunction, as well as endemic and relic, in some examples: *Helleborus*, *Incarvillea*, *Kelloggia*, *Thermospideae*, *Acanthochlamys*, *Meconopsis*, *Notholirion*, *Coriaria*, *Hippophae*, and *Arenaria*, showed that dominant modern floristic elements in Qinghai-Tibet Plateau such as Temperate of Old world, North temperate, Sino-Himalayan, Central Asia, and endemic were mostly origination from Tertiary Mediterranean flora. Furthermore, these analysis also demonstrated the dispersal pathway of the Tethys tertiary flora along Tethys.

2.7.1. From genetics to phylogenetics: shifting continents and paradigms in the evolution of seed-free vascular plants

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About 20 years ago, a revolution began in evolutionary studies of seed-free vascular plants. Whereas common wisdom averred that minute spores diminished barriers to long distance dispersal, genetic analyses demonstrated outcrossing breeding systems that require two spores for each successful migration event. Since that discovery, vicariance has taken on renewed significance, and new data on the recent origins of major groups require fresh consideration of biogeographic assumptions. The Polypodiaceae yield phylogenetic insights through an exemplar integration of DNA sequence analysis and biogeography. New evidence shows separate yet parallel radiations in the New and Old Worlds. By combining sequence data, continental drift, and a reassessment of morphological features, a sometimes radical realignment of generic boundaries is obtained. Subsequent presentations in this symposium further promote a paradigm shift in the systematics and biogeography of seed-free vascular plants.

2.7.2. Possible long distance dispersal of ferns between Madagascar and Southeast Asia

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Madagascar is well known as a large island containing many endemic plant species. During our attempts to find cryptic species using nucleotide sequence data of the *rbcL* gene, we collected members of the *Asplenium nidus* and *Hymenasplenium unilaterale* complexes widely from the Old World tropics including Madagascar. We found many cryptic species that have distinct *rbcL* sequences and are reproductively isolated as shown by crossing experiments. Even though we could find some amount of *rbcL* sequence variations in the cross-fertile plants from Southeast Asia, plant samples with the identical or similar *rbcL* sequences were detected from Madagascar. For example, *H. unilaterale* from Madagascar, Java, and Borneo, and those of *H. exisum* from Madagascar and Sumatra showed the same *rbcL* sequences, and *A. nidus* from Madagascar and Vietnam also had similar *rbcL* sequences. These results suggest long distance spore dispersal between Madagascar and Southeast Asia, which are at least 6000km apart.

2.7.3. Phylogeny of the fern genus *Elaphoglossum* (Elaphoglossaceae) and its implications for biogeography

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We performed phylogenetic analyses of *Elaphoglossum* using morphological characters and sequences from cpDNA regions. The sampling included species from the Neotropics and Paleotropics. The wide distribution of *Elaphoglossum* makes it appropriate to study floristic relationships between Africa/Madagascar and the Neotropics, and to test hypotheses about relative contributions of long-distance dispersal versus vicariance. Results show the Paleotropical species are polyphyletic and suggest multiple, independent long-distance events in three out of the four major clades recognized in the genus. Given this and the fact that 75% of the species occur in the Neotropics, it appears that *Elaphoglossum* migrated from the Neotropics to Africa. The distribution of the genus is examined in the Indian Ocean area and the Hawaiian islands. Preliminary studies postulate four separate colonization events of which a single introduction resulted in a radiation of six Hawaiian endemic

species, and in the Indian Ocean at least 13 colonization events are involved for explaining the distribution.

2.7.4. Molecular biogeography and origins of the Hawaiian fern flora

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We are conducting a phylofloristic investigation of the geographical origins of the fern flora of the Hawaiian Islands through the application of molecular phylogenetics. Groups that we are presently studying include *Hypolepis* (Dennstaedtiaceae), *Dryopteris* & *Nothoperanema* (Dryopteridaceae), *Cibotium* (Dicksoniaceae), *Diplopterygium* (Gleicheniaceae), and *Adenophorus*, *Grammitis*, & *Lellingeria* (Grammitidaceae). For each group we have developed explicit, testable hypotheses of their historical biogeography based on pre-existing ideas and/or our preliminary data. By comparing the results across groups, we are testing the broad hypothesis that diverse, unrelated lineages share common pathways of dispersal to the Hawaiian Islands. Preliminary results from several groups suggest that the jetstream serves as a major vehicle of spore dispersal from the Indo-Pacific region. Our results have important implications for the origins of all wind-dispersed groups of organisms in Hawaii and for the processes of community assembly.

2.7.5. Origin and diversification of African ferns with special emphasis on polygrammoid ferns

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A phylogenetic approach is applied to infer the relationships of African ferns belonging to the highly diverse polygrammoid fern lineage. Recent studies have revealed the global relationships of these ferns and found evidence for distinct New World and Old World clades within this fern lineage. African and Madagascan polygrammoid ferns are related either to the Neotropical clades or to predominantly Asiatic Old World clades. We study six different groups (1) to identify the relationships among species occurring in Africa and Madagascar, (2) to detect the relationships of Afro-Madagascan taxa to species occurring in the Neotropics and Asiatic tropics, and (3) to find evidence for colonization of or departing from Africa. An African/Madagascan clade with a sister clade widespread in Asia and Australasia is found in drynarioid ferns and in the genus *Platyterium*. Both clades also show a split into an African and a Madagascan subclade. Evidence for colonization of Africa by Neotropical taxa is found in the genera *Microgramma* and *Pleopeltis*.

2.7.6. The age of New Zealand's ferns

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New Zealand has long been geologically isolated, with its separation from Gondwana beginning some 80 million years ago. However, evidence is accumulating from both molecular and fossil studies that much of the New Zealand flora originated only recently, seemingly via long distance dispersal. To investigate the age of New Zealand's ferns, we applied molecular dating analyses to DNA sequence data from 30 monilophyte and lycophyte genera. Each sampled genus comprised a pair of one species from New Zealand and one from elsewhere. The reconstructed divergence ages for the majority of these genera were much less than 80 million years ago. These results will be discussed within the context of the uncertainty surrounding the geological history of the region, the New Zealand fossil fern record, and the testing of hypotheses of origins for these fern genera in New Zealand (i.e., long-distance dispersal versus vicariance; direction of dispersal; one versus multiple colonisation events).

2.8.1. Measuring selection and constraints: how phenotypic space is shaped

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Modern evolutionary theory is a complex ensemble of concepts and theoretical constructs, some of which are articulated in mathematical fashion, and others are expressed as narratives. Some of the major concepts include, of course, natural selection, genetic drift, adaptive landscapes, fitness, species concepts, etc. This set of theoretical statements is associated with a bewildering array of empirical methods, including observational and experimental ones, that are meant to further our understanding of evolution in natural populations. This talk examines some of the key concepts in evolutionary theory and how well (or not) they square with the methods commonly employed to study questions related to those concepts. It will emerge that in some cases we are in need of either a re-conceptualization of some of our key ideas, or of new empirical approaches, or both.

2.8.2. Moderator's comments: Understanding adaptation in plants

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Understanding adaptation in plants poses both conceptual and methodological challenges. First, defining a putatively adaptive trait requires a conceptual distinction between the advantageous and the historically/developmentally constrained aspects of any phenotypic feature. Second, although we often seek to test adaptation at the single-trait level, the adaptive value of any given trait is contingent on the suite of interacting traits that together constitute the phenotype. Similarly, the selective impact of any single environmental factor depends on the entire complex of environmental influences in the organism's natural setting, which is difficult to approximate experimentally. Finally, the measurement of fitness itself is far more complex than is indicated by the conventional reliance on total reproductive output. It is essential to consider additional traits that contribute to fitness in different ways, such as age at reproduction and quality of offspring.

2.8.3. Natural selection in relation to biotic and abiotic environments

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Studies in wild populations and in experimental settings have compared the form and strength of the natural selection across environments that differ in either biotic or abiotic conditions. Recently, researchers in the field of "community genetics" have argued that genetic variation in interacting species (e.g., competitors) can lead to reciprocal evolution of both partners. However, we know little about the relative force of selection via biotic vs. abiotic conditions. If selection via biotic factors is weaker or more variable in time or space than abiotic selection, species interactions may have little long-term effect on phenotypic evolution. I review studies published since 1996 on patterns of selection to address the following questions. Do biotic or abiotic conditions exert stronger selection on phenotypic traits? How does the strength of selection differ across environments that vary in biotic (e.g., presence or absence of competing species) vs. abiotic (e.g., wet vs. dry environments) conditions? How variable is selection via biotic vs. abiotic conditions in time and space?

2.8.4. The contribution of maternal effects to adaptation in natural plant populations

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Environmental maternal effects may evolve as a source of adaptive plasticity between generations. This concept will be illustrated using *Campanula americana*, an herb that grows in understory and light-gap habitats. Estimates of seed dispersal suggest that offspring typically experience the same light environment as their mother. In a field experiment testing the effect of open versus understory maternal light environments, maternal light directly influenced offspring germination rate and season, and indirectly affected germination season by altering maternal flowering time. Germination season determines whether

individuals grow as annuals or biennials, and results to date indicate that maternal effects on life history schedule are adaptive. Direct- and maternal-genetic variation for timing of germination (V_A and V_{Am}) will also be presented. In total, this provides a comprehensive assessment of the contribution of maternal light effects to life history evolution in this species. Evaluating maternal effects in an ecological context is necessary to demonstrate that they may provide phenotypic adaptation to local environments.

2.8.5. Issues in testing local adaptation - competition and environmental distance

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Local adaptation is usually tested in reciprocal transplant experiments calculating the home versus away contrasts of the site by provenance interaction. Often, experiments are conducted under reduced competition, i.e. in weeded monocultures or in vegetation disturbed by planting of seedlings. Furthermore, a home-site advantage is commonly used to recommend close-by populations in restoration schemes - regardless of the habitat type in which populations occur. We set up a large-scale reciprocal transplant experiment with Swiss, Czech and UK populations of three grassland species. The effect of competition was tested by comparing spaced individuals in weeded monocultures and plants sown together with ten typical grassland species. At each site a close-by population from a contrasting habitat was compared to the two foreign populations. The home vs. away contrasts were largely affected by the competition treatment indicating that local adaptation under natural conditions cannot be predicted from plants grown without competition. Close-by populations from a contrasting habitat were often more different to the home population than foreign ones.

2.8.6. Quantitative evolution in natural populations: neutral markers and chemical defences in *Eucalyptus tricarpa*

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Neutral genetic markers can be useful tools for studying the evolutionary ecology of adaptive traits in natural systems. Marker-based investigations of natural populations and open-pollinated genetic trials have provided unique insight into the causes of defence trait variation in *Eucalyptus*. Formyl phloroglucinol compounds are the most effective and well-characterised defences of eucalypts and protect trees from both mammalian and insect herbivores. However, the high level of within-population variation requires explanation in such ecologically significant traits. Marker-based heritability methods in the wild, regression of progeny phenotypes on wild parents and family reconstruction in open-pollinated seedlots were used to estimate natural quantitative genetic parameters. Despite substantial additive genetic variance, comparison of microsatellite divergence (F_{st}) with population differentiation in defence chemicals (Q_{st}) provides evidence for historical selective processes.

2.8.7. News in *Capsella* (Brassicaceae) - flowering ecotypes along a latitudinal transect from the Kola Peninsula (67°N) to Astrakhan (46°N) in Russia

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Capsella bursa-pastoris (L.) Medik. is a self compatible tetraploid annual species with high colonizing success in all parts of the world and is mostly confined to man-made habitats. One reason for the success is the finescale differentiation in flowering ecotypes. 18 Populations of *C. bursa-pastoris* have been collected along more than 2500 km length from the Kola Peninsula to the Caspian Sea. The transect includes short vegetation periods with extremely long days to hot and dry to salty conditions. The populations have been investigated in a common garden field experiment in order to differentiate between phenotypic plasticity and ecotypic differentiation of quantitative life history traits (flower begin, rosette diameter, leaf forms, plant height, branching and fruit characters). In addition, isozyme analyses have been performed. The most northern and most southern provenances belong to rather early flowering types, the provenances in between are medium to late flowering.

2.9.1. Response of northern forest trees to rising atmospheric CO₂ and tropospheric O₃: Mechanisms of altered forest productivity

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Atmospheric concentrations of CO₂ and O₃ have increased dramatically since pre-industrial time. Since the impacts of these two co-occurring pollutants are diametrically opposite and because relatively few long-term studies of trees exposed to elevated interacting CO₂+ O₃ have been done, it is difficult to predict how forest productivity will be affected as these two pollutants continue to increase in the atmosphere. For the past 7 years, the Aspen Free Air CO₂ Enrichment (FACE) project has been examining the impacts of these co-occurring pollutants for two pioneer tree species (aspen and birch) and a later successional species (maple). In addition, many of the same aspen genotypes from Aspen FACE have been examined along a natural O₃ gradient in the Lake States region over the past 10 years. In this presentation, mechanisms controlling forest tree growth and productivity for these three species and two studies will be described under long-term exposure to elevated CO₂ and/or O₃.

2.9.2. Responses of *Pinus sylvestris* seedlings from four European populations to a polar light regime in a CO₂-enriched atmosphere

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Ecosystem models predict the northwards migration of boreal forests over the next century as the climate warms and the atmospheric CO₂ concentration continues to rise. In future, therefore, boreal forests will experience a novel and poorly understood interaction between high CO₂ atmosphere and a polar light regime. We examined this interaction experimentally using four European ecotypes of *Pinus sylvestris* spanning a latitudinal transect from 40° to 68°N grown under polar (70 °N) light conditions with either ambient (400 ppm) or elevated CO₂ (800 ppm). Chlorophyll a fluorescence measurements indicated elevated CO₂ significantly enhanced photoinhibition in all four ecotypes at the growth irradiance (300 μmol PAR m⁻² s⁻¹), and after a short-term exposure to high irradiance (600 μmol PAR m⁻² s⁻¹), during the continuous light of the polar summer. In addition, we found no significant effects of elevated CO₂ on plant growth (height or stem diameter) in any of the four ecotypes. Thus, our results suggest that, at high latitudes, photoinhibition might offset the expected effects of CO₂ fertilization during *P. sylvestris* establishment.

2.9.3. Effects of season-long elevation of [O₃] on leaf growth and photosynthesis of soybean in the field

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Surface ozone concentration ([O₃]) has risen from an estimated pre-industrial value of 10 ppb to an average of ~60 ppb in the middle latitudes of the northern hemisphere, where it is predicted to rise by 20% in the next 50 years. The effects of elevated [O₃] (1.2 x ambient) on soybean (*Glycine max* cv. Spencer) leaf growth and photosynthesis were investigated under fully open-air conditions at the SoyFACE facility in Urbana-Champaign, IL, USA. The experiments were performed during pod-fill, when detrimental effects of O₃ would have the greatest impact on soybean yield. Photosynthetic carbon uptake was reduced in both growing and mature leaves by ~30% due to low stomatal conductance. Soluble leaf carbohydrate contents were lower under elevated [O₃]. Starch accumulation was observed in mature leaves, indicating altered sugar transport to sink organs. These changes in photosynthate availability appear to have reduced leaf growth rates, resulting in decreased leaf area at the end of pod-fill. However, the final yield was unaffected by elevated [O₃] suggesting photosynthate was preferentially allocated to reproductive sinks.

2.9.4. Pre-conditioning of alpine forest to tolerate ozone.

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The ozone impact on forest growth does not directly correlate with the tropospheric ozone concentration, as calculated via the tree-specific critical level (spruce: 10 ppm.h, AOT40), but it is supposed to correlate far better with the rate of O₃ uptake (influx) and the formation of reactive oxygen species (ROS). Ozone risk maps of Austrian forest, based on this concept, showed large areas of alpine forest with growth reductions of 30%. These calculated findings were not confirmed by the forest stock inventory and/or tree physiological studies on the spot. In contrast this research showed, that the ozone influx, controlled by leaf conductance (gL) did not appropriately modify the obvious response of alpine forests to ozone. Whereas, with increasing altitude, trees seem to be pre-conditioned to tolerate higher ozone levels. This pre-conditioning was probably achieved by the adaptation to i. the "pre-industrial" ozone concentrations and/or by ii. a long-lasting adaptation the exposure to UV-B depending on latitude and altitude.

2.9.5. Does leaf photosynthesis of plants originating from natural CO₂ springs adapt to CO₂-enriched environments?

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To explore adaptation of leaf photosynthesis to CO₂-enriched environments, plants originating from three natural CO₂ springs (spring-plants; mean habitat CO₂ was 500-1000 μmol mol⁻¹) and nearby control areas (control-plants; c. 370 μmol mol⁻¹) were grown at two CO₂ treatments (ambient CO₂: 370, elevated CO₂: 700 μmol mol⁻¹). We hypothesized that plants having a higher resource use efficiency at elevated CO₂ have been selected at natural CO₂ springs, because they may have higher competitive fitness in CO₂-enriched environments. This hypothesis was examined in the following three aspects: photosynthetic nitrogen use efficiency (PNUE), water use efficiency (WUE) and carbohydrate accumulation. The improvement of PNUE with a selective decrease in the amount of Rubisco, as predicted by theory, was not observed in all spring-plants. Lower stomatal conductance and higher WUE were found in spring-plants originating from one of the CO₂ springs, but not in those from the other two springs. Leaf starch accumulation tended to be lower in spring-plants originating from one of the CO₂ springs, which appeared to relieve down-regulation of photosynthesis.

2.9.6. A link between stomatal development and cuticular wax composition

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The *Arabidopsis thaliana* *HIC* (for *High CO₂*) gene encodes a putative fatty acid elongase believed to be involved in the synthesis of very long chain fatty acids. Plants carrying a mutation in the *HIC* gene or engineered such that *HIC* gene expression is reduced display an increased number of guard cells when grown under elevated concentrations of carbon dioxide (Gray *et al.*, Nature 1990). As fatty acid elongases are involved in the production of very long chain fatty acids, which are components of the cuticular waxes, the stomatal index of several wax deficient mutants was investigated. *cer1* and *cer6*, which have mutations in decarbonylase and fatty acid elongase encoding genes respectively, were shown to have abnormally high stomatal indices. These results indicate a link between the control of stomatal number and the biosynthesis of cuticular waxes. The results of recent experiments on the possible involvement of waxes in the control of epidermal cell development at elevated atmospheric CO₂ will be presented.

2.9.7. Elevated Carbon Dioxide Concentrations Inhibit Nitrate Assimilation in the Shoots of C₃ Plants

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Six independent methods affirmed that elevated CO₂ inhibits NO₃⁻ assimilation in shoots of C₃ plants (*Arabidopsis*, barley, *Flavaria pringlei*, giant redwood, tomato, and wheat), but not in NADP⁺-ME C₄ plants (maize and *Flavaria bidentis*). These methods examined growth and protein content, shoot CO₂ and O₂ fluxes, NO₃⁻ uptake vs. accumulation, mutants deficient in NO₃⁻ reductase, ¹⁵N discrimination, and ¹⁵N partitioning after a ¹⁵NO₃⁻ pulse. Several mechanisms may be responsible. 1) Photorespiration and the NADP⁺-ME C₄ pathway may increase malate levels in the cytoplasm to generate the NADH required for NO₃⁻ reduction. 2) Elevated bicarbonate levels may interfere with NO₂⁻ translocation into chloroplasts. 3) NO₂⁻ reductase may obtain sufficient ferredoxin only when the C₃ pathway is CO₂ limited. These results explain several responses of plants to rising CO₂ levels including CO₂ acclimation and CO₂ suppression of respiration. They also suggest that as atmospheric CO₂ levels increase, shifts in the relative use of NH₄⁺ and NO₃⁻ as N sources will influence the primary productivity of C₃ plants, thus altering species composition.

2.10.1. Growth and impact of measurements that do not perturb the plant. The example of tracer fluxes

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The annual number of publications using non-invasive techniques in plants rose exponentially from 1985 to 2000 and is still rising though more slowly. This parallels the increasing interest of investigators in functioning in the intact plant, in interactions between processes and in complex systems. A semi-systematic tabulation of non-invasive techniques will be considered in relation to major problems of plant biology in order to see what match there is. Tracer techniques for measuring fluxes and compartment contents in living cells will be reviewed in more detail. The critical considerations for applying a compartmental interpretation to a tracer exchange curve are: the system should be in a steady state, all compartments are fully mixed, there is no discrimination between tracer and traced ion or molecule. The experimenter must also ensure that the data cover the whole time course of exchange. Under these conditions it may be possible to estimate fluxes at plasmalemma and tonoplast.

2.10.2. Effect of nutrient stress on algae: a species comparison by FTIR-spectroscopy

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The identification of nutrient limitation of phytoplankton growth is of considerable importance to our understanding of the ecology of aquatic systems. In addition, water quality of freshwater systems is controlled by the availability of nutrients limiting the aquatic primary productivity. As a response to nutrient stress phytoplankton cells undergo metabolic acclimation resulting in shifts of total C, N, and P quota. This was shown for e.g. nitrogen, phosphate, and silica limitation of algae. We employed Fourier Transform Infrared (FTIR) spectroscopy as a non-invasive technique to study changes in spectral features of several algae in response to nutrient stress. Monitoring changes of spectral band ratios and correlating the ratios with actual cellular C:N and C:Si quotients allowed the detection of species-specific shifts. Since the growth rate is known to depend on C, N, and P quota, FTIR can not only be used to detect the type of stress, but also its influence on growth and the competition capability of species. As an example FTIR spectroscopy was used to identify N-limitation of *Microcystis* sp. in a hypertrophic lake.

2.10.3. Relationships between leaf shape, leaf temperature, carbon gain and water use in South African Pelargonium species

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There is a tremendous range of variation in leaf shape among plants and we are only beginning to understand its functional significance. Theoretically, a divided or dissected leaf should track

ambient temperatures more closely than an entire leaf of the same area. Because photosynthesis is optimum at a certain temperature, differences in leaf temperature will affect carbon gain. Transpiration also increases with temperature, thus, leaf-level water-use-efficiency (WUE) declines once temperature exceeds the optimum for carbon gain. We measured the thermal response of photosynthesis in Pelargonium plants grown at two temperature and water levels and asked whether leaves from species with different shapes varied in gas exchange traits. Results show that plants acclimated to growth temperatures and had very broad optimal temperature ranges. Thermal optima exceeded growth temperatures under both regimes, but optimum for WUE was significantly lower. Notably, dissected leaves had higher photosynthetic capacities than entire leaves, we propose that this may reflect a suite of correlated traits associating leaf shape, morphology and physiology.

2.10.4. Emergent Behavior and Information Processing by Stomatal Networks

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Stomata must respond to environmental factors such that they open to admit enough CO₂ for photosynthesis, yet close sufficiently to prevent excessive water loss. Much of this regulation occurs at the level of the individual pore, through signal transduction pathways in the guard cells. However there is increasing evidence that stomata interact with each other over short distances and can therefore be said to form a locally-connected network. Chlorophyll fluorescence images and thermography can be used to show that stomatal conductance can often be patchy and that stomata can behave collectively. This collective behavior may indicate that stomatal networks are processing information in a manner similar to artificial networks that perform distributed emergent computation. This information processing may allow stomata networks to optimize gas exchange for an entire leaf or plant despite the fact that each individual stoma has only local information.

2.10.5. Non-invasive analysis of water and carbon transport and plant growth by nuclear magnetic resonance and positron emission tomography

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Plant growth and transport processes are highly dynamic. They are characterized by plant-internal control processes and by strong interactions with the spatially and temporally varying environment. Analysis of structure-function relations of growth and transport in plants will strongly benefit from the development of non-invasive techniques. The paper will describe recent advances in the low- and high-field NMR imaging of plant transport in xylem and phloem as well as on root growth analysis in artificial soils. Non-invasive functional analysis of the dynamics of carbon transport is possible by using the short-lived isotope ¹¹C. In addition to standard detection techniques, the recently developed positron emission tomography system for plants (PlanTIS) allows imaging the flow of ¹¹C-labelled compounds in two and three dimensions. These techniques will provide a real insight into the dynamic nature of growth and transport processes of plants in variable environments.

2.10.6. Development of uniformly stable isotope labeling system in higher plants for hetero-nuclear NMR experiments *in vitro* and *in vivo*

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Novel methods for measurement of living systems are making new breakthroughs in life science. In the era of the metabolome (analysis of all measurable metabolites), a MS-based approach is considered to be the major technology, whereas a NMR-based method is recognized as minor technology due to its low sensitivity. Therefore, my laboratory is currently focusing to develop novel methodologies for an NMR-based metabolomics. This will be achieved by uniform stable isotope labeling of higher plants allowing application of multi-dimensional NMR experiments used in protein structure determination [1]. Using these novel methods, I will analyse the dynamic molecular networks inside tissues. Especially, use of stable isotope labeling methods has

enormous advantage for discrimination of incorporated or *de novo* synthesized compounds. Furthermore, potentiality of *in vivo*-NMR metabolomics will be discussed in the conference. [1] "Stable isotope labeling of *Arabidopsis thaliana* for an NMR-based metabolomics approach." *Plant Cell Physiol.* **45**, 1099-1104 (2004).

2.11.1. Noise-sustained patterns in excitable systems.

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The ability of excitable systems to generate and propagate patterns, or coherent structures, is well known. This property has been widely demonstrated in biological, chemical, physical as well as in ecological systems. In all such systems an inherent threshold mediates the excitation and propagation of the structures. In the absence of random fluctuations, or noise, structures can propagate only when generated by an excitation that exceeds the threshold. But when the excitation is sub threshold, the structures propagate only a short distance and then die out. It is nevertheless observed that sub threshold structures can propagate over long distances in the presence of noise. Such systems are modeled as networks of coupled nonlinear oscillators throughout which supra threshold and noise sustained sub threshold coherent patterns can propagate. Coupled noisy oscillators also exhibit stochastic phase synchronization, a closely related topic. Examples from a chemical, neural and biological system are presented.

2.11.2. Spatio-temporal pattern formation of photosynthesis from leaves to the canopy - do spatial variations really matter?

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Light use efficiency of photosynthesis dynamically adapts to a variety of internal and environmental factors often rendering photosynthesis heterogeneously distributed over single leaves or organs. These spatio-temporal variations reflect regulatory properties of photosynthesis and may have a constructive role for stability in metabolic pathways and during development. Natural canopies are complex 3-dimensional structures, exposed to a fluctuating light environment. The tropical rainforest mesocosm within the Biosphere 2 Center has been subjected to a series of 4-6-week drought treatments. Ecosystem CO₂ uptake rate declined 32% and ecosystem transpiration declined 50% in response to the drought, reflecting both morphological and physiological responses, including increased leaf fall, reduced maximum photosynthetic electron transport rate, and stomatal closure. Individual trees responded differently, expressing different levels of stress avoiding mechanisms. This functional diversity renders the individual response heterogeneous and has implications to scale leaf level responses to the dynamics of the „big leaf“ canopy.

2.11.3. Metabolite snapshot analysis reveals correlative biological fluctuation as a result of constant environmental perturbation in plant systems

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For the analysis of plant systems we developed a technological platform based on snapshot metabolite profiling. For comprehensive analysis of representative compound classes gas chromatography coupled to time of flight mass spectrometry was used. Analytical precision is in the range of 10-30 % coefficient of variation. Biological fluctuation of independent samples exceeds this threshold several fold [1]. The metabolite variances are used for multivariate data analysis. Pairwise metabolite correlations are extracted from the data set and analysed with respect to correlation network topologies [2]. These dynamic changes in correlation networks are connected to underlying pathway networks [3, 4]. Numerical analysis of calvin cycle and sucrose metabolic pathways in leaf metabolism predict distinct metabolite correlation networks in dependence of environmental parameters and enzyme regulation. [1] Weckwerth et al. *Proteomics* 2004 [2] Weckwerth et al. *PNAS* 2004 [3] Weckwerth *Annual Review of Plant Biology* 2003 [4] Steuer et al. *Bioinformatics* 2003

2.11.4. Coupling of different oscillators in the circadian rhythm of Crassulacean acid metabolism (CAM)

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In continuous light, the Crassulacean acid metabolism plant *Kalanchoë daigremontiana* undergoes a circadian rhythm of gas exchange. This rhythm, however, is not paralleled by the expression of CAM rhythmicity (i.e. storage and release of malate). Instead, a substantial proportion of the rhythm's amplitude is accounted for by C3 mediated uptake of CO₂. Experiments applying thermoperiodic entrainment have revealed the existence of an oscillator driving stomatal conductance which functions separately from the oscillator controlling CO₂ uptake by the mesophyll (Bohn et al. 2003 *Biol. Chem.* **384**: 721-728). When stomatal conductance was eliminated by coating leaves with silicone grease, the mesophyll level rhythmicity was still expressed. Experimental analysis of the gas exchange rhythm in *Kalanchoë* has revealed a complexity of oscillating systems, including controls of CAM and C3 carboxylation pathways, as well as stomatal conductance working in a network to produce the overt CO₂ uptake rhythm.

2.11.5. "Functioning-dependent structures" (FDSs) in higher plants and fungi

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Proteins involved in metabolic/signalling pathways are sometimes organised into multimolecular assemblies ranging from quasi-static complexes to dynamic associations only forming in an activity-dependent manner. These exist in all sorts of organisms and include the ATP- and pH-dependent association/dissociation of the V₁ and V₀ domains of the yeast vacuolar H⁺-ATPases and the nucleotide-dependent pentameric/hexameric transition of the head structure of a higher plant V-type H⁺-ATPase. We term *functioning-dependent structure (FDS)* any multimolecular structure in which proteins (or other molecules) associate due to the very fact that they are in the process of accomplishing their function and from which they dissociate when ceasing to function. Apart from the well-known advantages of protein assemblies compared with free proteins (better resistance to hydrolysis, channelling of intermediates), our modelling shows that, under steady state conditions, FDSs may display kinetic properties such as a prolonged linear response or a sigmoidal behaviour, even when their constitutive proteins do not display such properties when functioning separately.

2.11.6. Non-linear dynamics of plant photosynthesis in fluctuating light

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Variable cloud cover and moving canopy are among the dynamic factors that have been shaping the photosynthetic regulation since the first ancestors of modern plants emerged. Plants developed regulatory feedbacks to sustain homeostasis of plant bioenergetics in fluctuating light. These feedbacks are responsible for emergent, systemic properties of plants that are observed in harmonically modulated irradiance. The non-linear, upper harmonic modulation of fluorescence emission and of CO₂ assimilation are measurable signatures of the dynamic regulation of photosynthesis during forced oscillations. We use the forced oscillations and frequency analysis of the reporter signals for systems identification of the photosynthetic regulatory motifs. An open-access, web-based modeling project aiming at this goal is presented. Molecular mechanisms with a potential of causing the non-linear dynamics are discussed. In parallel, we show that the forced oscillations and the analysis of the upper harmonic modulation of fluorescence emission can be used to detect regulatory mutants and plant segments affected by a biotic stress.

2.11.7. Dynamical complexity of gas exchanges and higher network connectance improves homeostasis of plants under water deficit

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We hypothesized that more irregular or complex temporal dynamics and a more interconnected overall network supports greater stability to gas exchange parameters (herein, CO₂ net assimilation and transpiration) in plants under water deficit. To test this hypothesis two genotypes of *Phaseolus vulgaris* were subjected to a period of absence of irrigation, and subsequent rewatering to achieve recovery. Gas exchanges parameters were measured each 10 s during 6 h to obtain time series to evaluate complexity by Approximate Entropy (ApEn) calculations, and network connectance in each water regime. Notably, the Jalo Precoce genotype showed significantly more stability than the Guarumbé genotype under system perturbation, coincident with greater irregularity in each gas exchange parameter and greater overall connectance for Jalo. This conclusion is consistent with other observations of greater homeostasis in more complex networks, seen in broad contexts such as cardiac rhythms and respiratory dynamics.

2.12.1. Molecular systematics and chemotaxonomy: The importance of molecular phylogeny to interpret phytochemical relationships

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The occurrence of secondary metabolites (SM) often agrees with taxonomic groups and has therefore been used to infer systematic relationships; even a new discipline “chemotaxonomy” had been established. This concept was based on the assumption that SM had no function and that they would strictly reflect phylogenetic relationships. However, presently we regard SM as defence and signal compounds, important for the plant's survival and reproductive fitness. SM therefore represent adaptive characters that have been subject to natural selection. A closer look into the distribution of SM reveals that certain metabolites are absent (or present) in a given taxon, although all the neighbouring and ancestral taxa express or (do not express, respectively) the particular trait. The inconsistent SM profiles mean that the systematic value of chemical characters becomes a matter of interpretation in the same way as traditional morphological markers. Their occurrence apparently reflects adaptations and particular life strategies embedded in a given phylogenetic framework.

2.12.2. Review on taxonomy of the genus *Murraya* (Rutaceae) from chemosystematic viewpoints

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The genus *Murraya* belongs to the orange subfamily (Aurantioideae) in Rutaceae, and comprises two sections: *Murraya* and *Bergera*. Section *Murraya* contains 4 species and 3 varieties, all of which are closely related each other from taxonomical viewpoints. *M. paniculata* is geographically the most wide-spread species of Section *Murraya* occurring in either the tropics or subtropics of Asia and Oceania. This species has found wide medicinal value throughout the area of distribution. Its ethnobotanical use varies outstandingly from one area to another, which is presumed to reflect botanical diversity of the origin at not only the infra-specific but also specific level. We have thus been induced to undertake intensive chemical investigation on this species of various localities, and have clarified chemical diversity of this species. A series of chemical studies revealed that *M. paniculata* should be divided into two species and several chemical races depending upon the types of prenylcoumarins contained. This paper deals with taxonomical review of both Section *Murraya* and genus *Murraya* from viewpoints of both morphology-based taxonomy and chemotaxonomy.

2.12.3. The systematic significance of isoflavonoids in the *Lecointea* clade (Leguminosae: Papilionoideae: Swartzieae).

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Despite many morphological similarities, some molecular phylogenies indicated that the *Lecointea* clade, a basal group of Leguminosae, Papilionoideae, is not natural, but a recent molecular work suggested that this group is monophyletic. This paper aims to verify if the detection of flavonoids can be helpful in the classification of these Leguminosae taxa to see with which of the phylogenies the chemical data corroborate. For that it was accomplished a phytochemical study in roots of *Harleyodendron unifoliolatum* R.S.Cowan, *Lecointea hatschbachii* Barneby and *Holocalyx balansae* Micheli. This study resulted in the isolation of sixteen isoflavonoids. We detected four pterocarpanes (two unpublished), three isoflavans and two isoflavanones (both are also unpublished) in *Harleyodendron unifoliolatum*; one pterocarpan, one isoflavone (unpublished) and three cumestans (two unpublished) in *Lecointea hatschbachii*; and two isoflavanones in *Holocalyx balansae* (also unpublished). The phytochemical evidences indicate that the members of *Lecointea* group have flavonoids and isoflavonoids that are not found in any member of the *Vatairea* group.

2.12.4. Using molecular phylogenies of the genus *Veronica* s. str. to assess iridoid and flavonoid characters

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Early systematic classifications of the genus *Veronica* s. str. (fam. Plantaginaceae, formerly Scrophulariaceae) were mainly based on macromorphological features such as the position of the inflorescence. Subsequent karyological, seed morphological and phytochemical studies suggested that these classifications did not reflect the phylogenetic relationships within the genus. Recently several molecular studies have been carried out which have led to a new phylogenetic intrageneric classification of *Veronica*. This has provided us with a framework to assess the phylogenetic value of chemical characters for the classification of the genus. For example, unusual 8-hydroxyflavone glycosides, which according to previous classifications seemed to occur in unrelated groups in the genus, now appear to be characteristic of the new subgenera *Pocilla* and *Pentasepalae*, which are sister groups in the molecular phylogenetic analyses. Likewise, the iridoid glucoside mussaenoside appears to be a good phylogenetic character for the new subgenus *Veronica*.

2.12.5. Secondary metabolites in relation to molecular phylogenies in *Aloe* and related genera (family Asphodelaceae)

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Chemotaxonomic studies of *Aloe* and related genera of the family Asphodelaceae over the last 40 years have revealed a remarkable complexity of secondary metabolites. Most genera accumulate tetrahydroanthracenones in the roots and these metabolites show interesting discontinuities at generic level. Knipholone and related compounds appear to be confined to *Bulbine*, *Bulbinella* and *Kniphofia*, while asphodeline and related compounds are more widely distributed. 1-Methyl-8-hydroxyanthraquinones are present in the roots of most *Aloe* species (with further oxidation in *Gasteria*), while the leaves have anthrone-C-glycosides, chromones, phenylpyrones and rarely flavonoids. Nectar sugars are uniform within genera but show diagnostic differences amongst genera. General trends, congruence and inconsistencies in the presence of secondary metabolites will be highlighted, with reference to recently published molecular phylogenies. The overall pattern supports the hypothesis that hybridization must have played an important role in the phylogenetic history of the subfamily Aloioideae.

2.12.6. Mapping of the distribution pattern of terpenoids on a molecular phylogeny inferred from ITS sequence data in *Nepeta* L.

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The compositions of essential oils in *Nepeta* have been studied by GC-MS. The presence of terpenoids was interpreted on a molecular phylogeny based on the analysis of nucleotide sequences of the nrITS. The main components of the essential oil in most *Nepeta* species are monoterpenoids, 1,8-cineole and nepetalactone are major compounds, but rarely sesquiterpenoids are predominant. The results show that some of the monophyletic groups in the molecular phylogeny share the same class of compounds, for example, species of Sects. *Capituliferae*, *Micranthae* p.p. and *Micronepeta* p.p. are characterized with 1,8-cineol, transpinocarveol, pinocarvone, myrtenol and myrtenal. The species of Sect. *Spicatae* are characterized by nepetalactone and 1,8-cineol as the major constituents, sabinene and beta-pinene in lower quantity. The species of sects. *Nepeta*, *Micranthae* p.p. and *Macrostegiae* p.p. are characterized by nepetalactone as the major constituent and a considerable number of sesquiterpenoids. In the species of Sect. *Psilonepeta* sesquiterpenoids are more frequent than monoterpenoids. The results provided data for a new infra-generic classification of *Nepeta*.

2.12.7. Sesquiterpenoids as chemosystematic markers in the subtribe Hypochaeridinae (Lactuceae, Asteraceae)

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Molecular analyses are currently the gold standard within biosystematic research. However, many of the clades in phylogenies arising from molecular analyses are only weakly characterized by morphological data. In such instances, phytochemical data might help in phenetically defining clades found in molecular investigations. In contrast to the delimitation of the Lactuceae tribe from other tribes of the Asteraceae family, which is well established on the basis of morphological, phytochemical, and molecular markers, the borders of genera within the Lactuceae are not undisputed. Recent molecular analyses indicate that current generic limits within the Hypochaeridinae result in paraphyletic groupings. In this communication sesquiterpenoids are employed to phenetically characterize undisputed supra-specific entities within the subtribe Hypochaeridinae (Lactuceae, Asteraceae). The phytochemical data are congruent with recent molecular results and imply e.g. that the genus *Leontodon* will have to be divided into two separate genera.

2.13.1. Plant functional types as tools to bridge across scales

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Plant functional research has soared for the last ten years as a result of the impetus given by global change questions. Recent research has explored the significance of plant functional traits to environmental response and ecosystem effects. One first achievement has been the production of standardised lists of the most significant and easily measurable traits. Based on these, and on large efforts to synthesise the literature, it has been possible to identify and explain plant functional response traits associated with response to resource gradients (esp. nutrients) and widespread disturbances such as grazing and fire. Current research is focussing on the challenge of linking these to effects on community structure and on biogeochemistry, showing the relevance of fundamental trade-offs that constrain the way plant manage their resources. These considerable advances will now allow us to refine functional classifications used in dynamic vegetation models, as well as contributing to the debate on the relationships between plant diversity and ecosystem function.

2.13.2. Scaling from traits to communities to ecosystems: the challenge of complexity at intermediate scales

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Despite the potential importance of community dynamics, predictions of ecosystem responses to environmental changes are still often based on simple aggregations of individual plant responses. Regional-scale models often chose to ignore population and community-level phenomena on the grounds that this increased complexity does not add understanding. When does scaling need to incorporate community dynamics and when can it skip this level? Although higher-level responses to changing species and functional composition may often be modest and gradual, ignoring these dynamics may sometimes introduce large errors at higher levels of organization. We consider when it is necessary to deal explicitly with plant functional dynamics at the community level. First we outline the basic premises and assumptions of trait-based scaling, then present evidence that community-level phenomenon should be better incorporated into scaling approaches, and finally point to places where further research could help us better understand the importance of complexity at intermediate scales.

2.13.3. Can plant functional traits account for plant-plant interactions?

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Much research has been devoted toward understanding how individuals of co-occurring plant species interact and how these interactions influence structure and dynamics of communities. Reviewing studies assessing inter-specific competition phenomena, a list of intuitive questions asked by researchers and experimental procedures employed to address these questions is proposed. Two groups of studies emerge: "individual-centered" studies characterizing short-term changes in performance and proportions of species; "community-centered" studies documenting species establishment and existence in communities. A trait-based approach is proposed to link "individual-" and "community-centered" approaches. Effect traits acting on competitive intensity, are traits accounting for changes in local environmental conditions due to plant functioning. Examples of functional traits, involved in resource use trade-off, are given. Response traits characterize plant response to those environmental changes. They are related to plant growth, reproduction and longevity. Their relevance to assess changes in community structure and dynamics is discussed.

2.13.4. Response of plant functional traits to land-use changes in European mountain grasslands

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Land-use changes affect both the composition and the diversity of species in mountain grasslands. The related changes in plant functional traits, rather than species numbers *per se*, can be considered as major determinants of ecosystem biogeochemical cycles. Nine functional traits of more than 120 vascular plant species in 19 differently managed mountain grasslands across Europe were analysed as regards (i) their response to land use, (ii) their variation (i.e. the functional diversity of the ecosystems), and (iii) the definition of plant functional types and their relation to land use. It was found that with decreasing intensity of land management specific leaf area and mass-based leaf nitrogen concentrations (N_L) decreased, whilst leaf mass ratio and area-based N_L increased. The variation of canopy height, a measure of spatial heterogeneity, changed with land use and was closely related to the variation of plant height and carbon/nitrogen ratios, but not of biomass and leaf area. Based on a cluster analysis five plant functional types were distinguished, whose proportional

contribution to the different grasslands varied in response to land use.

2.13.5. Generic functional descriptors as a basis for scale-free vegetation classification and analysis

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A generic set of plant functional attributes and their elements is used to classify a plant as a unified functioning individual in terms of chlorophyll distribution, life form and aboveground rooting features. Any vascular plant can be readily described by combining the functional elements according to a specified rule set. While more than seven million combinations are theoretically possible, data from 1760 sites (40x5m transects) worldwide, suggests the real figure for the world's 250,000+ vascular plant species may be less than 4,000. Combinations of leaf adaptive features and supporting life form reflect both short and long-term plant response to environmental change and thus facilitate bridging scales of ecological analysis. Environmental, gradient-based case studies covering most of the world's key vegetation types are used to illustrate how data can be readily aggregated or disaggregated to target management or research needs at any specified environmental scale. Compared with purely species-based approaches, the method facilitates more meaningful cross-site ecological comparison and an improved basis for biodiversity assessment.

2.13.6. Plant functional traits capture changes in ecosystem functioning in response to land use changes

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Although the structure and composition of plant communities is known to influence the functioning of ecosystems, there is as yet no agreement as to how these should be described from a functional perspective. We tested the biomass ratio hypothesis, which postulates that ecosystem properties should depend on species traits and on species contribution to the total biomass of the community, in 11 sites across Europe. These sites have undergone various land use changes, leading to changes in species composition and functional characteristics. Preliminary results suggest that ecosystem primary productivity and litter decomposition rate varied significantly with disturbance regime and availability of nutrients in several sites, and that these were correlated with community-aggregated - *i.e.* weighed according to the relative abundance of species - functional traits. We argue that some of the easily measurable traits tested provide simple means to scale-up from organ to ecosystem functioning in complex plant communities. These can be used to assess the impacts of community changes on ecosystem properties induced, in particular, by global change drivers.

2.13.7. Developing a general framework of rare plant population responses to habitat fragmentation

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To effectively manage plant populations for conservation, there is a need to provide reliable information on the conditions required for maintaining viable populations. This is particularly true for rare taxa occurring in a fragmented landscape, such as that of south-west Western Australia. Resources do not exist to undertake comprehensive studies of population dynamics for every rare plant species of this region. By assigning rare plant taxa into functional groups, based on their floral architecture and putative pollinator, their fire life history and germination requirements, models will be developed for each functional group. Taxa were chosen from each functional group for detailed studies on how rates of pollination and seed production are affected by population size and landscape context. The results of these models will be extrapolated to other taxa within each functional group thereby providing guidelines for flora conservation, threatened ecological communities, and restoration programs. Here we present findings on our allocation of the rare plant taxa of WA into floral functional groups and how these groups respond to landscape fragmentation.

2.14.1. Microphytobenthos: a tool for monitoring, or a subject of ecological research?

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Communities of diatoms and other microphytes are currently used for the routine monitoring of water quality. The EU-Water Framework Directive stipulates the use of microphytobenthos as a yard stick for ecological quality. It is questioned here whether the information on spatial distribution of species can be linked to specific biological attributes and to defined causes of environmental change. Microphytobenthic consortia develop structures embedding species, biofilms, and these provide a battleground for inter-specific competition. These properties of biofilms may confound one to one relationships of species attributes and distribution in environmental gradients. Environmental factors such as salinity or pH may not produce large discrepancies, yet experimental proof is lacking. Nutrients and essential metals are put forward as factors steering species composition via coupled effects on different levels of molecules up to biofilm communities. Research approaches will be discussed, using a number of case studies.

2.14.2. Probation of lifetime express-methods of control for functional state of Algae under water contamination

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The aim of investigations was selection of biophysical methods, making quick information possible on living algae cells. In experiments had been used natural and synthetic surfactants. From methods have been checked laser-doppler spectroscopy (LDS), extremely high frequency dielectrometry (EHF-dielectrometry), differential fluorimetry (DF) and luminescent-microscopic methods. As objects were investigated 17 species of algae from *Cyanobacteria*, *Chlorophyta*, *Rhodophyta*. LDS makes possible the estimation of speed of cells movement ($\mu\text{m/s}$) and its energy potential of moving (relative units). EHF-dielectrometry method makes possible the estimation of hydration level for intracellular structures of algae and state of water (free or bound) in it. For estimation of rates of biomass growth the exact results are obtained by DF method at the sacrifice of determining not only the ratio for different algae sections (by concentration of chlorophyll *a* and presence of other pigments) but also index *F* (level of its potential photosynthetic activity). It was proved that one of these lifetime nondestructive methods may be used for express-control.

2.14.3. Using algal assessments and ecological thresholds to develop environmental criteria

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Numeric environmental criteria provide resource managers with specific targets for protecting or restoring valued ecological attributes. Non-linear relationships between valued attributes and pollution help justify specific concentrations of contaminants for criteria. Non-linear responses of ecological systems to environmental change should be common, but are seldom observed in large-scale studies. Temporal variability and scale of observation can be refined to detect thresholds in ecological response. Diatom indicators of total phosphorus concentrations of waters more precisely infer phosphorus conditions than one-time sampling and assay of total phosphorus in studies of both streams and wetlands, where nutrients have high temporal variability. Diatom indicators provided a temporally integrated assessment of phosphorus that increased the resolution in non-linear macroalgae-nutrient responses, suggesting that diatom indicators were more accurate measures of phosphorus availability than total phosphorus concentrations. Thresholds in algal-phosphorus relations provided candidates for numeric nutrient criteria.

2.14.4. An experimental approach to refining ecological optima and diatom species concepts

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Whatever the organisms used, biomonitoring relies upon reliable identification of taxa that have well-defined ecological ranges and optima. However, for the majority of diatoms, ecological ranges and optima are based on relative abundances in field material

correlated with physical and/or measurements, and a morphological species concept prevails, based on the interpretation of field collections. It is usually assumed that morphology is constant across environmental conditions, and that intra-specific variation is minimal. However, experimental studies and the occurrence of Janus cells have shown that diatom morphology is not constant across all conditions, but may vary in response to environmental variables. Similarly, there are inherent assumptions in the use of relative abundance data to calculate ecological optima that may falsify the results. The use of culture studies can address both issues. This paper will describe how species concepts and ecological optima can be refined using an experimental approach.

2.14.5. Recent developments in the use of benthic algae for ecological water quality analysis in rivers of Central Europe

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Extensive databases of benthic macro- and microalgae collected from a wide variety of lotic habitats and bioregions are used successfully for biology based water quality analysis in Central Europe. Especially for the mountain areas the bioregion and river type specific approaches (using data on species composition and dominance) allowed the clear identification of trophic status beside other environmental distortions (e.g. changes of hydraulic pattern). In these areas the identification of near-natural reference situations (as requested by the WFD) is still possible, whereas in most lowland areas reference situations are rare. For the latter cases reference situations can partly be reconstructed from historical data (herbaria, samples, documentations etc.). These data combined with biotests of species responses enables the biologist expert to establish a plausible system to identify degradation grades of natural communities; however it is a matter of politics to identify minimum requirements at which action must be taken to improve undesirable situations.

2.14.6. Floristical and seasonal distribution of epiphytic algae in Anzali Lagoon, Iran

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This study was carried on epiphytic algae in Anzali lagoon (Gilan province, Iran) from September 2000 through August 2001. Epiphytic algae were removed from natural and artificial substrates. Five macrophytes were used as natural substrate and dowel wooden rod as artificial substrate. In this study 150 algae were identified and enumerated by using Sedgwick-Rafter cell. Species belong to 6 classes and 64 genera. Species of Bacillariophyceae with 27 genera and 95 species were dominant and had the highest diversity. Chlorophyceae with 21 genera and 29 species was next. Cyanophyceae with 10 genera and 15 species. Euglenophyceae with 4 genera and 9 species and Dinophyceae and Crysophyceae represented with one species each. We used SIMI (Sanders Similarity Index) and CC (Community Coefficient) to compare community structure and relative similarity of epiphytic algae between different substrates which revealed that epiphytic algal communities were not restricted to a specific substrate. Algal biomass on artificial substrate was higher than natural substrates. This might be due to inhibitory compounds secreted by plants such as allelopathic substances.

2.14.7. The biotesting of aquatic environment using test-systems - microalga and bioluminescent bacteria.

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Using three different biological test-systems: green microalga *Scenedesmus quadricauda* Breb., *Daphnia magna* Straus. and express-detection - bacteria bioluminescent test-system "Ecolum-08" (*Escherichia coli* K12 TG1 with cloned lux-operon of *Photobacterium luminescens* ZM1) the influence of low-intensive electromagnetic field (EHF) on aquatic environment toxicity was studied. It is shown, that extremely high frequency irradiation can increase or diminish toxicity water solutions of phenol, pesticides, cadmium, cobalt, copper ions and their mixture. The effect

observed depends on intensity, dose of EHF and on the type of substance and its concentration. To estimate toxicity we used microalga survival and express-detection (30 minutes) of bioluminescence intensity of bacteria. The possibility to diminish aquatic environment toxicity under EHF-irradiation seems to be very promising for practical use in ecology regulation.

2.15.1. Building a sustainable world

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We live in a world of 6.4 billion people, half of them in poverty and malnourished, and at least 500 million literally starving. The global population might stabilize at 9 billion in a few decades if we continue to deal with it effectively. At present, however, we are estimated to be consuming global productivity at about 120% of the rate at which it occurs, up from an estimated 70% in 1970. We view with alarm the potential extinction of more than two-thirds of all species of eukaryotic organisms on earth by the end of the century, and must take steps to avoid this serious loss. The intensification of sustainable agriculture on productive lands is likely to be the most effective single strategy that we can undertake to accomplish this goal, using among other techniques biotechnology, integrated pest management, and the controlled use of water and chemicals to produce the desired results.

2.15.2. Development of transgenic temperate fruit trees and grapevines for virus resistance

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Different traits have been modified in transgenic fruit trees, comprising altered processing and storage qualities, modified nutritional properties, i.e. the influence of desirable/ undesirable components, modified growth habit and vigor, resistance to biotic and abiotic stresses, e.g. drought, low temperature. The breeding and cultivation of virus resistant plants is a major contribution to the control of viral diseases, since there do not exist chemical control strategies. However, beyond technical feasibility, efficiency of resistance, environmental safety and IPRs, also public acceptance needs to be considered. Also access to IP was defined as a major obstacle in the development of transgenic fruit and vegetable crops. Efforts are required to create public understanding and acceptance for these crop plants. To build public confidence the project "Characterisation of transgenic fruit trees and analyses of direct and indirect biological interactions" was initiated to demonstrate the step-by-step principle with GMOs (<http://www.boku.ac.at/sicherheitsforschung/open-e.htm>), supported by the BMBWK and BMLFUW, Austria.

2.15.3. Government policies & public perception of biotechnology

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In an increasingly global and knowledge-intensive economy, the ability to manage impacts of new technologies is an essential skill of governments and industry. New technologies introduce new uncertainties and potential risks related not only to human health and the environment but also to industrial competitiveness and trade. It is because of this that governments respond to the introduction of biotechnology in different ways. Governmental policies, in general, reflect the influence of a combination of factors the most prominent of which are: • socio-economic considerations • scientific evidence • the remit of regulatory institutions The fact that the priorities of, and therefore public acceptance of biotechnology in the industrialised world and least developing countries differ should be acknowledged. However, while such differences do exist, they have to be respected and ways be found that lead to greater convergence of mutual interests.

2.15.4. Environmental health aspects in modern food production

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Whereas farming has contributed to creating and maintaining a variety of valuable semi-natural habitats, consequences of food production have also contributed to pollution, resource depletion

and loss of habitats, the main hazards for biodiversity according to the Convention of Biological Diversity (CBD). The results of the Millennium Ecosystem Assessment (MA) recently provided alarming evidences on the failures to achieve the UN- Millennium Development Goals and the severe loss of ecosystem diversity, where services of ecosystems are the basis for human health and well being. New developments in food production, such as technologies using modern methods of biotechnology as well as technologies educated more by ecological sciences including IPM or organic farming will ultimately need to avoid and correct dangerous developments. For GM food production international regulations, the CODEX Guidelines and the Cartagena Protocol on Biosafety have been implemented. Research and comprehensive reports such as the CEC- report on effects of transgenic maize in Mexico significantly increased knowledge and the quality of the risk assessment, but also specified areas of concern such as the need for better molecular characterisation and improved methods for profiling, gene flow and indirect consequences of changes in farming practices enabled by new methods. The values for protection in the environment are often debated differently, reflecting also regional different agro-ecological baselines and concepts of conservation biology. Coherent analysis suggests that local specific agro-ecological or even socio-economic characteristics often determine if a specific agricultural practice is beneficial in an area and models for a decision of the best possible practice in a specific area have been proposed. UN- organisations furthermore, demand improved forms of public private partnerships in developing, shaping, integrating and accessing to modern technologies to ensure equitable sharing of benefits, a central element of the CBD and sustainable development.

2.15.5. The unfinished agenda in agriculture

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We have an unfinished agenda. We also have an unfinished debate about what will be best for each country if it is to avoid hunger catastrophes in the future. This is the foremost task of this workshop, to find solutions. And the purpose of the session is to show you that actually there are many ways and many roads that give us solutions. 1. I am against eco- and corporate imperialism. More decisions should be made by countries and regions on their own. 2 Organic farming and high-tech farming need to go together in the future. We simply have no time for ideological debates and battles over these issues. We should install a collaborative learning process instead. So, let's benefit from the advantages and avoid the disadvantages. And clearly we need to monitor new and old technologies and establish regulatory frameworks, which balance out risks and benefits. 4. In the near future we should focus on the UN Millennium Project, which will reduce dramatically famine for some 400 million people by 2015 - if only we find the political will to take some decisions and avoid unnecessary ideological fights in agriculture.

2.16.1. Metabolic engineering: a general background to the analysis of metabolic networks

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Metabolic engineering depends on knowledge about the structure of metabolic pathways. This includes the structure of pathways themselves, information about sites where flux is controlled, and information about regulatory networks regulating the key enzymes. It has become increasingly clear that changes in the expression of single enzyme do not always generate the expected result. Sometimes this occurs because an inappropriate enzyme has been selected. I will consider strategies for identifying key regulatory enzymes. Frequently, changes in expression are compensated for by adjustments in the regulatory network. Thus it is sometimes more effective to alter the levels of regulatory metabolites or the regulatory properties of enzymes, rather than the level of expression of the enzymes themselves. However, control of flux is often shared between several enzymes in a pathway. It can then be useful to alter the regulatory networks in order to alter the expression of all or most of the enzymes in a pathway. I will consider how the distribution of control in metabolic pathways can be analysed, and give examples of the use of overexpressed transcription factors to alter metabolic fluxes. Often, unexpected interactions also lead to unexpected or undesired changes. To understand these responses, knowledge about the

structure of the metabolic network is required. These can be analyzed using forward and reverse genetics, and more recently by the use of functional genomics. It will be discussed how multilevel genomics analysis using near whole-genome 22K ATH1 arrays for routine expression profiling, an in-house platform that allows sensitive and quantitative profiling over >1200 transcription factors by multiplexed real time RT-PCR, a robotised platform for the measurement of enzyme activities, and robotized enzymic assays and metabolite profiling can be used to understand complex metabolic responses.

2.16.2. Metabolic engineering of nitrogen assimilation with the Dof1 transcription factor in higher plants

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Nitrogen assimilation is essential to the growth and development of plants. We investigated the utility of a plant-specific transcription factor, Dof1, in modifying nitrogen assimilation in higher plants, because Dof1 appeared to be an activator for multiple gene expressions associated with the organic acid pathway providing carbon skeletons for nitrogen assimilation. Expressing Dof1 induced up-regulation of genes encoding enzymes for carbon skeleton production, a marked increase of amino acid contents, and a reduction of the glucose level in transgenic *Arabidopsis*. In addition, elementary analysis revealed that the nitrogen content increased in the Dof1 transgenic plants. Most significantly, the Dof1 transgenic plants exhibited improved growth under low-nitrogen conditions, an agronomically important trait. An increase in the amount of free amino acids was also observed in the transgenic potato plants expressing Dof1. These results suggest that key transcription factors modulating regulons are powerful tools for metabolic engineering in higher plants.

2.16.3. Photosynthetic assimilation of formaldehyde in plants by genetic engineering

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Most plants are photoautotrophic organisms mainly using CO₂ as a carbon source. Formaldehyde (HCHO) is a ubiquitous air pollutants and is also very toxic for plants. On the other hand, HCHO is a key intermediate for biological fixation of C1 compounds in methylotrophs. 3-hexulose-6-phosphate synthase (HPS) and 6-phospho-3-hexuloisomerase (PHI) are two important enzymes in HCHO fixation pathway in many methylotrophic bacteria. Our study shows that a functional photosynthetic assimilation pathway for HCHO can be installed by over-expression of HPS and PHI genes from a methylotrophic bacterium in the chloroplasts of *Arabidopsis thaliana* and tobacco. The expression of the two genes enhanced the tolerance of the transgenic plants to HCHO and augmented their ability to take up either gaseous or aqueous HCHO. Tracer experiments showed that H¹⁴CCHO was assimilated into non-volatile metabolites and the TCA-insoluble fraction. This creates a novel strategy for phytoremediation of HCHO pollutant. This study might also open the way to confer plants an ability of photosynthetic assimilation of the other C1 compounds such as methanol and methane.

2.16.4. A biotechnological approach to improve the efficiency of CO₂ assimilation: re-cycling of glycolate in chloroplasts of *Arabidopsis thaliana*

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In C3 plants the carbon gain by photosynthesis is tightly coupled to CO₂ loss by photorespiration starting with the oxygenation of RubP catalyzed by RubisCO. The prime function of this first reaction of the C2-photosynthetic carbon cycle is to salvage glycolate-2-P by conversion to glycerate-3-P, which re-enters the C3-reductive cycle. In C3-plants, competition of O₂ with CO₂ at the active site of RubisCO results in a loss of at least 25% of the fixed carbon. Hence, diminished photorespiration might, in principle, contribute to an increased biomass production in C3-plants. It was therefore attempted to transfer the advantages of C4-photosynthesis to C3-plants. Häusler et al. have shown that the overexpression of C4-cycle genes in C3-plants leads to an attenuation of

photorespiration, but also to pleiotropic changes in the pattern of endogenous enzymes. We have introduced two alternative and complete glycolate catabolic cycles into chloroplasts of the *A. thaliana* in order to release and re-fix CO₂ directly within the chloroplast. Analyses of single and double transformants and those with fully operational glycolate cycles will be presented.

2.16.5. Metabolic engineering of natural products for nutritional enhancement

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The impact of plant natural products on human health is increasingly recognized. However, only with the advent of genetic and genomic approaches has their biosynthesis been understood sufficiently to permit their engineering in crop plants. Proanthocyanidins (PAs) are oligomeric flavonoid antioxidants derived from (-)-epicatechin and/or (+)-catechin. Co-expression of the enzyme anthocyanidin reductase with flavonoid pathway transcription factors can introduce epicatechin into tissues that do not normally make it. Soy isoflavone phytoestrogens can be engineered in a number of species that naturally lack the pathway; the level of production depends on the activity of endogenous competing pathways, and can be increased by blocking these pathways. Engineered isoflavones accumulate as different sugar conjugates depending on the plant species. Saponins are components of a wide variety of herbal medicines. Genomics approaches are leading to the discovery of the genes encoding the enzymes for the biosynthesis of this complex family of glycosylated plant terpenoids, and metabolic engineering of the pathway is underway.

2.16.6. Turning blue - Commercializing metabolic diversions in flowers

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Genetic Engineering is a tool of great potential for the ornamental plant industry with numerous examples of genetic manipulation reported in the literature. Typically these involve the use of model systems some of which have limited direct commercial value. However, when applied to commercial crops there arises the possibility of creating novel products - the driver of the ornamental industry. To date there is only one ornamental GM product, a carnation genetically engineered for novel flower colour, in the marketplace. These carnations were developed under a joint venture between Florigene Ltd and Suntory Ltd. The novel coloured flowers produced are on sale in the USA, Japan, Australia and some countries of Europe. Other species are now entering the commercialization pipeline. The research, development and commercialization of carnation and rose are summarized. As the long term prospects for ornamental GM products, like food crops, is determined by the regulatory environment the commercialisation of our flowers will be looked at with reference to the current regulations in market and production territories and the specific genetic modifications made.

2.16.7. How safe is the chloroplast?

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It is often suggested that placing transgenes in the chloroplast in genetically modified plants will offer significant protection in the field against transmission of the transgenes through the pollen, because chloroplasts are only very rarely transmitted (if at all) through the pollen of most crop plants. However, if chloroplast DNA were transferred to the nucleus at a significant frequency, this containment would be breached. It is clear that, on an evolutionary timescale, there has been major transfer of genes from the chloroplast to the nucleus. It has also been known for some time that movement from mitochondrion to the nucleus takes place at an unexpectedly high frequency in *Saccharomyces cerevisiae*. Recent studies have shown that transfer from chloroplast to nucleus also takes place at a significant frequency in tobacco. However, experiments using the green alga *Chlamydomonas reinhardtii* failed to detect transfer. This may provide information on the mechanism of transfer.

2.17.1. Europe's contribution to facilitating biodiversity data access for users

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The European Network for Biodiversity Information, supported by the European Commission, has as its general objective the development of an open network of biodiversity information centres in Europe including national nodes of GBIF. The network is coordinated by the University of Amsterdam and has 60 members in 24 countries. The Network is opening up biodiversity information present in European institutes which hold the richest and most important biodiversity collections, literature and observation data and is concentrating on databases and activities that need co-operation at a European level, that bring together emerging initiatives in biodiversity informatics and analysis tools of connected databases, and is exploring the design of potential new research infrastructures that build upon the databases. The future organisation of such infrastructures at the national and international level is being discussed. Much of the initial information and opinion has come via the use of e conferencing and workshops. ENBI will focus on the market of end-users with special attention to developing specific products and services for professionals and policy makers.

2.17.2. From ink to electrons - Challenges and solutions in digitising herbaria

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Collectively, the plant and animal specimens in the world's museum collections combined with recent observational and monitoring data provide our most complete picture of the biological diversity of the planet. Unfortunately, the vast majority of this valuable legacy data is generally underutilized because it is not easily accessible in a digital format. The challenge is how do we cost effectively and efficiently digitally capture, interpret and make accessible this vast underutilized information resource? Due to the vast amount of legacy data residing in the world's herbaria, even small increases in efficiencies can make significant differences in overall costs. In January 2005, ENBI and GBIF co-hosted a workshop that brought together a group of international experts to discuss how recent advances in information technologies could be applied to increase the speed and efficiency of capturing the label data associated with the millions of specimens in the world's herbaria. The outcome of this meeting and its associated recommendations will be discussed.

2.17.3. Analysis of digital plant specimen data: the example of Gabon

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In a joint effort coordinated at Wageningen, data on 95% of all herbarium specimens of vascular plants ever collected in Gabon has been digitised (BRAHMS software). The over 60,000 records provide an extremely powerful source of information to be used for research on and conservation and sustainable use of the vegetation in a country that harbours the richest lowland rain forest in Africa. An analysis of the data shows a.o. that, despite the intensified collecting efforts in the last 3 decades, only 16 ¼ degree squares are botanically fairly well known (>1 specimen per km²). The total number of known species (native or escaped after introduction) is c. 5100 in 1342 genera and 192 families with the level of endemism being 10%. Numbers are still increasing every year, leading to an estimated true species richness of c. 6500. Using the correlation between number of specimens and species richness, an estimate of the total species richness per ¼ degree square can be made by extrapolation. Certain areas prove to be of particular importance for conservation. Methods to arrive at a biodiversity score for each square using a rarity-weighted species richness index are discussed.

2.17.4. Two decades of the ILDIS project and its World Database of Legumes

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The International Legume Database & Information Service (ILDIS) is an international project started in 1985. Regional centres in 12 countries and a network of 48 taxonomic experts are participating to pool information in the ILDIS World Database of Legumes, which is used to provide a worldwide information service through electronic access, publications and enquiry services. The latest edition (2005) contains a near to complete World Species Checklist of the *Fabaceae* / *Leguminosae* family (c. 20,000 species). An on-line service is provided via LegumeWeb (www.ildis.org), Species 2000 & ITIS Catalogue of Life (www.sp2000.org) and GBIF Data Portal (www.gbif.org). Major ILDIS publications include 6 check-lists published at the Royal Botanic Gardens Kew: Legumes of Africa (1989), Legumes of West Asia (1991), Legumes of Indo-China (1994), Legumes of Northern Eurasia (1996), Legumes of South Asia (2003), Legumes of Malesia (2004); Phytochemical Dictionary of the Leguminosae (1994) and two CD-ROMs: Legumes of Northern Eurasia (1998) & ILDIS Legumes of the World, a draft checklist (2001). ILDIS has been supported by the GBIF ECAT programme in 2004/2005.

2.17.5. Flora Croatica database

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Flora Croatica Database (FCD) is a multi-user database application for species-oriented and specimen-oriented systematic and taxonomic work on Croatian flora. It is designed for dealing with all kinds of data that are commonly used in systematic botany and floristic work. FCD comprises several main modules: (1) taxonomy, (2) herbarium, (3) literature, (4) horology (5) economic botany and (5) related data (ecology, multimedia). FCD was built over a relational database and relies on the normalised data model. Amongst other features, the client application provides the user with extended query by example (QBE) capabilities and with user-customised reports. The reports include taxon sheets, tax checklists, herbarium labels, bibliography labels and other complex reports. The database is connected to a geographical information system (GIS) and FCD MapServer, which empowers easy production of distribution maps and other spatial analysis. Web interface enable searching, as well as data input, for several user types, together with geocoding facility and complex analysis of floral compositions.

2.17.6. Development of the database on economic plants of Flora of Armenia

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Flora of Armenia comprises about 3500 species of plants. Armenians have a long tradition of making good use of plant biodiversity for economic purposes. The analysis of botanic, entomological and other sources of information revealed that local population have used about 2000 species of 60% of flora in food, medicine, coloring, etc. In spite of rich diversity of plant species, botanic resources are limited. The development of database on economic plants is necessary for conservation biodiversity and sustainable use of botanic resources. Internet, the technology which gives an interface to data management via network capabilities, was selected as a technical solution for development such database. This method gives possibility to have public database, which could be connected without any additional installation software or hardware. Just an access to Internet. This web based software allows unlimited connections to database which is helpful for synchronous usage of the same data system. The database of economic plants contains detailed information

about them, their arrangement in subject catalogue as well as their usage and geographic location.

3.1.1. Moving with the flow: What transport reveals about cell division and expansion

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Recently there has been an explosion in the discovery of gene products associated with cell division. Several transport laws are useful to illuminate the physiological significance of the genes and proteins. Continuity equations reveal relationships among cell size and the rates of cell division and cell expansion and have been used with data on cell size and growth velocity to find cell division rates in three directions. Growth trajectories, functions showing the time course of the intra-organ position of cellular particles, can be used to find relationships among time, the appearance of gene products and the occurrence of cell division in control and genetically modified plants. Lagrangian specifications show correlations among cell division and later expansion rates. For these analyses, growth data in the meristem is needed. For root-soil interactions, a growth-diffusion equation can show the intra-rhizosphere pattern of metabolites produced or consumed during cell division.

3.1.2. The integration of cell proliferation and growth in leaf morphogenesis

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Tremendous advances have been made in our understanding of the patterns of transcriptional regulators that control leaf formation. However, the mechanism by which these patterns are transduced into actual change of form remain obscure. Intuitively, patterns of cell division must play a key role in this process but various strands of evidence indicate that this is not the case.

To investigate the relative role of cell growth and division on plant morphogenesis, we have developed a novel method (microinduction) to locally and transiently manipulate these cellular parameters, both in the shoot apical meristem and within the developing leaf primordium. Our data show that local modulation of cell wall extensibility is sufficient to induce leaf formation and to modify leaf shape. Local promotion of cell proliferation has no impact on leaf initiation and, surprisingly, leads to an overall decrease in growth of the leaf lamina. These data support the primacy of cell wall characteristics in controlling plant morphogenesis and indicate that the influence of cell division on morphogenesis is context dependent and non-intuitive.

3.1.3. Establishing and maintaining axial growth: wall mechanical properties and the cytoskeleton

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Organ morphology depends on cell placement and directional cell expansion. The microtubule cytoskeleton is implicated in both of these processes so genetic approaches to understand the role microtubules play in organ expansion are not straight forward. Our use of temperature-sensitive *mor1* mutants to disrupt cortical microtubule arrays in anisotropically expanding walls led to the surprising discovery that arabidopsis root cells can establish and maintain transverse cellulose microfibril texture without well organized microtubule arrays. One explanation for the altered mechanical properties of cell walls in these left-twisting and radially swelling mutants is that microtubule disruption affects the cell's ability to generate long cellulose microfibrils, which may be essential for perfect anisotropy. This new model opens up an attractive mechanism for the regulation of organ shape that depends not only on the orientation but also on the relative length of cellulose microfibrils to define the growth axis.

3.1.4. Crystal structure and evolution of expansins and their role in modifying the growing cell wall

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Expansins are proteins that loosen cell walls by dissociating wall polysaccharides, thereby allowing polymer creep and turgor-driven wall extension. They are important for plant cell growth and other

processes where wall loosening occurs (see <http://www.bio.psu.edu/expansins/>). Expansins are encoded by four families, with 36 members in *Arabidopsis* and 58 in rice. By microsynteny analysis combined with sequence-based phylogenetic analysis, we estimate that 16-17 expansin genes were present in the ancestor of monocots and eudicots. We will present a hypothetical scheme to account for family evolution since this time. To investigate expansin's mechanism of wall loosening, we crystallized native Zea m 1, a beta-expansin from maize pollen, and determined its structure by X-ray crystallography. We will describe expansin structure and how it might interact with the cell wall to cause loosening.

3.1.5. The *Arabidopsis* leaf as a model system to investigate the link between cell cycle regulation and whole organ growth

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Organ growth results from the progression of component cells through subsequent phases of proliferation and expansion before reaching maturity. We combined kinematic analysis, flowcytometry and microarray analysis to characterize cell cycle regulation during the growth process of leaves 1 and 2 of *Arabidopsis thaliana*. Kinematic analysis showed that the epidermis proliferates until day 12, thereafter cells expand until day 19 when leaves reach maturity. Flowcytometry revealed that endoreduplication occurs from the time cell division rates decline until the end of cell expansion. Genome-wide micro-array analysis allowed us to categorize known cell cycle genes into three major classes: Constitutively expressed, proliferative and inhibitory. Comparison with published expression data obtained from root zones corresponding to similar developmental stages and from synchronized cell cultures supported this categorization and enabled us to identify a high confidence set of 131 proliferation genes. Most of those were M-phase specific. Besides known cell cycle genes there were many unknowns or previously not associated with proliferation.

3.1.6. Establishing growth maps for *Antirrhinum* and *Arabidopsis* leaves

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In the past few years, many molecular biology studies have explored the role of genes in cell fate determination or regional patterning. However, understanding how these genes influence the dynamics of organ growth and shape change remains a major challenge. We want to bridge this gap by using a dynamic and integrative approach to model leaf growth in *Antirrhinum* and *Arabidopsis*. To do so, we have been using a combination of genetic, morphological, computational and imaging approaches. We have used tracking and clonal analysis of leaf cells, along with newly developed software, to analyse growth and obtain some growth parameters. This approach should provide clues to the primordial events triggering shape change and morphogen action. The talk will illustrate how integrating biological and computational methods may lead to a quantitative mechanistic framework for development and the possible implications for leaf mutant analysis.

3.1.7. Coordination of cell proliferation and cell expansion in the control of leaf size in *Arabidopsis thaliana*

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Leaf size is an important parameter characterizing leaf morphology. To understand the mechanisms controlling leaf size, we have isolated many mutants of *Arabidopsis thaliana* with altered leaf size. Since leaf morphogenesis depends on determinate cell proliferation, the size of a mature leaf is expressed by multiplication of cell size and cell number. Therefore, any leaf size mutants should be classified according to the specific effects of the mutation on the cell number and/or size. One of the classes suggests a mechanism that coordinates cell proliferation and cell expansion in developing leaves: when the leaf cell number decreases by a mutation such as *angustifolia3* (*an3*) and *aintegumenta*, the leaf cell size increases, leading to the compensation of leaf area. As the first step to elucidate the

mechanisms of compensation, we should understand the inductive step of compensation. To this end, cell number in the *an3* leaves was manipulated by crossing known or newly isolated cell-number mutants and its effects on the compensation were evaluated. Our results provide a novel key concept that explains how leaf size is controlled at an organ level.

3.2.1. Evolution of floral organ identity: a primer

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According to the ABCE and floral quartet models of flower development in higher eudicots, four classes of floral homeotic MADS-domain proteins interact in a combinatorial way to specify the identity of the four different types of floral organs, with class A+E proteins specifying sepals in the first whorl of the flower, A+B+E petals in the second whorl, B+C+E stamens in the third whorl, and C+E carpels in the fourth whorl. Flower development thus provides exciting opportunities to better understand the evolution of a morphogenetic system at the genetic and physico-chemical level. Comparative studies on orthologues of floral homeotic genes in phylogenetically informative seed plants, including all major groups of extant gymnosperms, basal angiosperms, diverse monocots as well as basal and higher eudicots, are currently providing detailed insights into the meaning, conservation and flexibility of floral organ identity. This again helps us to much better understand the origin and tremendous diversification of one of nature's most marvellous key innovations: the angiosperm flower.

3.2.2. Genomics of the lower eudicot *Aquilegia*: a new model for the study of floral evolution and ecology

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The angiosperm genus *Aquilegia* presents a unique opportunity to investigate the molecular genetic changes underlying a recent adaptive radiation that involved floral and ecological diversification. In addition, *Aquilegia* has the benefit of representing a phylogenetic lineage that is intermediate between core eudicot model species like *Arabidopsis* and monocot models, such as rice. This combination of assets (and small ~350 Mb genome size) places *Aquilegia* in the singular position of facilitating studies of both recent and ancient evolutionary processes. By the application of innovative genomic techniques, we are developing a wide array of tools that will enable a whole community of researchers to study adaptation at the genomic level. These essential tools include the development of finely mapped RILs, a physical map, an EST database, and oligonucleotide arrays. Progress in this project and the application of these tools to questions relating to floral evolution will be discussed.

3.2.3. Floral gene expression in basal angiosperms: Alternatives to the ABC model

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The ABC model of floral organ identity explains the regular, sequential development of sepals, petals, stamens, and carpels in eudicot flowers. This general model, based on studies of the derived eudicots *Arabidopsis* and *Antirrhinum*, may apply to nearly all eudicots, most of which are characterized by discrete whorls of floral organs. However, floral morphology of basal angiosperms is typically characterized by variable numbers of floral parts and gradual transitions among floral organs. We have investigated patterns of expression of A-, B-, C-, and E-function homologues in a sample of phylogenetically important basal angiosperms: *Amborella*, *Nuphar*, *Illicium*, *Magnolia*, *Eupomatia*, *Asimina*, and *Persea*. Homologues of floral genes from *Arabidopsis* and *Antirrhinum* are expressed more broadly across the floral meristem of basal angiosperms than in these models. These broader patterns of gene expression correspond to gradual intergradations among floral organs. Furthermore, broad patterns of expression are inferred to be the ancestral condition in angiosperms, followed by restricted localization of gene expression in core eudicots.

3.2.4. Evolution of floral homeotic genes in green plants

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The flower is a reproductive organ in angiosperms, whose development is mainly managed by MADS-box genes and their positive regulator FLO/LFY genes. We characterized these genes in the basal angiosperms (*Nymphaea*, *Magnolia*, *Houttuynia*, and *Amborella*), the gymnosperm (*Gnetum*), the fern (*Ceratopteris*), the moss (*Physcomitrella*), and three charophycean green algae. These analyses suggest that (1) MADS-box genes were originally involved in haploid reproductive cell differentiation, (2) MADS-box genes were recruited in the diploid development early in land plant evolution, (3) the induction of FLO/LFY gene of MADS-box genes was established during vascular plant evolution after divergence from mosses, (4) Involvement of B-function MADS-box genes in petal development was established at the early stage of angiosperm evolution, (5) antagonistic expression of AP1 and AG orthologs was not established early in angiosperm evolution.

3.2.5. Floral organ identities and expression of MADS-box genes in Magnoliales

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To investigate the floral organ identity programs in basal angiosperms with highly divergent floral morphologies, we examined expression patterns of floral MADS-box genes in representatives of Magnoliales. *Magnolia* flowers have an undifferentiated perianth of spirally arranged tepals. In contrast, *Asimina* has whorled, trimerous flowers with a perianth of distinct sepals and petals. *Eupomatia* flowers appear to lack a perianth but bear a calyptra, the homology of which is uncertain. Data from relative-quantitative-RT PCR, real-time PCR, and RNA *in situ* hybridization suggested that (1) broad expression of B-class homologs across the floral meristem in *Magnolia* correlates with the development of petaloid tepals; (2) organ identity in *Asimina* is controlled by a mechanism that is similar to, but independently derived from, that of *Arabidopsis*; and (3) the calyptra of *Eupomatia* exhibits gene expression patterns consistent with its interpretation as a modified bract. These results suggest that the ancestral floral developmental program was labile, with shifts in gene expression leading to profound differences in floral morphology, even in a single clade.

3.2.6. The evolution of carpel development.

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Molecular phylogenetic analyses have identified the ANITA grade species to very probably represent the earliest diverging lineages of flowering plants. Concurrently, excellent progress is being made in understanding the molecular control of flower development in a few highly-evolved model species. A comparison of basal and model angiosperms conducts us back in time to their (very early) common ancestors. By this method, it may be possible to formulate an anatomical and molecular picture of flower development in the common ancestor of the flowering plants. We are using comparative genomic techniques to identify genes from model plants whose roles in the development of the carpel, or female reproductive organ, have been conserved since the common ancestor of the angiosperms. Some of our recent studies have identified likely conserved functions in carpel development for the orthologues of *CRABS CLAW* and *TOUSLED* in the ANITA grade species *Amborella trichopoda* and *Cabomba aquatica*, respectively. We are also working to establish *C. aquatica* as a model angiosperm in which gene functional analyses will be possible.

3.2.7. Floral developmental genetics and genomics in *Gerbera* (Asteraceae)

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The development of inflorescences in the plant family Asteraceae has features that cannot be easily studied in the traditional model plants for flower development. In *Gerbera hybrida*, inflorescences are composed of morphologically different types of flowers tightly packed into a flower head (capitulum). The special features of

Gerbera inflorescences have made the plant a rewarding target for comparative studies. We have shown that *Gerbera* MADS box genes grouping phylogenetically with B and C function genes of *Arabidopsis* and snapdragon function in organ determination very similarly than in the model plants. However, MADS box genes encoding proteins that interact with those of the ABC MADS box genes are different. For example, a *Gerbera SEPALLATA*-like gene controls not only organ identity, but also inflorescence architecture and reproductive meristem fate. A particularly intriguing question in Asteraceae relates to genes that regulate different development in marginal and central flowers. We are currently exploring differences in gene expression of *Gerbera* flower primordia using a cDNA microarray based on a collection of 16,994 *Gerbera* EST sequences.

3.3.1. In search of the primitive monocot flower: insights from comparative and developmental morphology

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Recent molecular studies have clarified our understanding of higher-level monocot relationships, but evolution of morphological characters, including flower morphology, remains problematic. Parsimonious optimisation of floral characters onto existing molecular phylogenies suggests primitiveness of the "typical" monocot flower groundplan, i.e., six tepals in two whorls, six stamens in two whorls and three fused carpels in a superior ovary. This hypothesis involves reversals to non-monomerous apocarpny in some Alismatales, Pandanales and Arecaceae, which contradicts the traditional view that apocarpny is primitive. Monocot flowers with apocarpny also differ in other respects from the typical monocot flower groundplan, for example, in (functional) unisexuality and/or variation in merosity. This suggests that either (1) "typical" monocot floral structure evolved many times in parallel, or (2) in some monocot lineages a partial loss of integrity in regulation of flower development resulted in loss of the monocot floral groundplan.

3.3.2. Evolution of flowers and inflorescences in Monocotyledons

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The orientation of flowers is constant in many families, e.g. the median sepal of the outer whorl is always precisely adaxial in Eriocaulaceae and abaxial in Xyridaceae. In several families of the Liliales and Asparagales the position of the flower is highly variable. Classical phyllotactic patterns describe how the position of a leaf follows preceding leaves on the same axis and do not explain the position of the first leaf of a lateral axis. We found good evidence, that in species with variable orientation of the flowers, the flowers represent reduced lateral racemes. The orientation of the flowers shifts from being influenced by the lateral axis to being defined by the main axis. The variability in floral orientation is a last remainder of a previously more complex inflorescence. The shift of the prophyll in many monocot inflorescences from an adorsed position to a more transversal position resembling the situation in dicotyledons may be the effect of very short internodes at initiation of the lateral branches. If the internode is still elongated when the axilar bud is formed, the prophyll is forced into an adorsed position by the pherophyll (subtending leaf).

3.3.3. Floral evolution in a highly derived monocot clade, Poales

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Improved phylogenetic resolution among the 18 constituent families of Poales, including identification of *Ecdiocollea* as a potential sister-group to grasses, allows new assessments of floral evolution in this economically important and species-rich order. Establishment of a developmental genetics programme in grasses has improved the potential for recognition of genes controlling specific floral traits. Despite these advances, the enigmatic homologies of grass reproductive structures remain poorly understood, partly because their highly derived morphology has resulted in a unique terminology. Two of the three families that form the basal grade of Poales, Bromeliaceae and Rapateaceae, have retained some of the characteristic aspects of the monocot floral groundplan such as showy tepals and septal nectaries, while

the third, Typhaceae, displays anomalous and highly reduced floral morphology. The remaining four lineages (graminids, restiids, cyperids, xyrids), which all include abiotically-pollinated species, have undergone homoplastic evolution of traits such as perianth reduction and loss, and reductions in ovule and carpel number.

3.3.4. Some ontogeny and a little biology of floral diversification in the Commelinaceae

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The Commelinaceae rival the orchids with respect to the diversity of floral forms and complexity. Floral diversification within the family has proceeded primarily via reduction, fusion, and various elaborations upon the plesiomorphic plan of six free stamens with longitudinally dehiscent anthers. Ontogenetic studies show this diversity to be underlain by a greater degree of ontogenetic diversity (i.e., similar flowers can be formed by different ontogenies). Examples include the occurrence of either centrifugal or centripetal patterns of stamen development to produce structurally similar androecia. Additionally, presumed homologous patterns of stamen reduction are achieved via either early abortion or absolute suppression of stamen primordia. Although my emphasis is on ontogeny, floral biology will be reviewed, and connections between the two will be highlighted with an example of the evolution of buzz-pollination in the family via very different morphogenetic pathways. This last case study also illustrates the process of reciprocal illumination between both ontogenetic and phylogenetic studies in regards to questions of structural homology.

3.3.5. Comparative floral structure and systematics of *Pelagodoxa* and *Sommieria* (Arecaceae)

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Floral structure is compared in two genera of Arecoideae and discussed in a phylogenetic framework. Male flowers have three free, imbricate sepals, three basally congenitally united and apically valvate petals, and six stamens. Anthers are dorsifixed and introrse. The sterile gynoecium is tricarpellate. Female flowers have three free, imbricate sepals and three free, imbricate petals, which are fused with the sepals at the base. Four to six staminodes are congenitally united at the base and partially fused with the ovary. The gynoecium is syncarpous and pseudomonomerous. The three stigmatic branches are apical and sessile. The carpels are (syn-)ascidiate up to the level of the placenta and (sym-)plicate above. Each carpel has one ovule. The fertile ovule is erect up to anthesis and pendant afterwards. Pollen tube transmitting tracts encompass the secretory epidermis of the ventral slits. Floral structure in *Pelagodoxa* and *Sommieria* supports the sister group relationship between the two genera suggested in recent molecular phylogenies and reflects their close relationships to a major clade of arecoid palms from the Indo-Pacific region.

3.3.6. Pollination syndromes and diversification rates in the tropical *Gingers* (Zingiberales)

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The development of novel structures is a major force in the diversification of plant lineages. Within angiosperms, floral structure presents a variety of traits upon which evolutionary forces act to create novel forms and functions. Many of these traits are associated with the specialization of pollination. In the Zingiberales, functional pollinators include mammals, birds and insects. Shifts between functional pollinator groups is linked with changes in the form of floral characters that reflect pollinator association. Using a phylogenetic framework and whole-tree methods for determining differential diversification, we demonstrate that shifts in major pollination syndromes are correlated with shifts in rates of diversification, indicating that floral syndrome acts as a key innovation in the evolution of diversity in tropical gingers. The main floral organ involved in pollination is the stamen, which develops a petal-like form in four of the eight major lineages. In two of those lineages (Costaceae, Zingiberaceae), 2 -

5 stamen fuse to form a novel structure, the labellum. The role of the labellum in the diversification of the Zingiberales is discussed.

3.3.7. Floral transitions among orchids: from the sublime to the ridiculous

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Orchidaceae probably originated from an ancestor with radially symmetrical flowers. Two alternating whorls of three tepals occurred below two alternating whorls of three stamens, all closely spaced and connected to an inferior trilocular ovary. In contrast, strong bilateral symmetry characterises the majority of floral whorls of most extant orchid species. The evolutionary route leading to species-rich orchid groups required well-developed epigyny, progressive partial or complete suppression of the adaxial stamens, and fusion of the remaining stamen filaments to a syncarpous style; this generated a gynostemium. Subsequent evolutionary divergences relied more upon differentiation between (a) the two whorls of tepals and (b) the median (labellum) and lateral members of the inner tepal whorl. Exceptional variation is evident in tepal shape, colour and outgrowths such as spurs; these interact with the detailed morphology of the gynostemium to dictate pollination biology. We will review the limited data on the evolutionary-developmental genetic controls of such transitions, and explore evidence for their relative frequency, rapidity and profundity.

3.4.1. Biomimetics - taking a leaf from Nature's book

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Introductions inspire, preparing the listener by reviewing the topic, illustrating thoughts which lead to success, then challenging with ideas and conundrums to which those thoughts have not yet been applied. The listener is thus prepared for the rest of the presentations, receptive to the science and ready to ask searching questions. Look through this list, check the topics you know about, and think of ways of using them. Then work out what the other topics are going to be, and how the botanical examples can be turned from curiosities into technical marvels. Remember - one of the basic skills of creativity is making lists! Materials [Tough materials (wood analogues); Surface properties (Lotus, *Nepenthes*)] Structures [Attachment (Velcro, ivy, climbing *Galium*); Transpiration (textiles); Light-weight structures; Space and supportive structures (*Ficus*)] Mechanisms [Deployable structures (unfolding, growth, fungi); Energy storage (legume pods, cucurbita); Water sorption (sensing and actuation); Growth and Repair (*Pisum*)] Self- [Design and adaptation (cellular automata); Sensing (Venus fly trap)]

3.4.2. Purity of sacred Lotus: self-cleaning super-hydrophobic micro- and nano-structured biological surfaces and their biomimetic technical analogues

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Our discovery of self-cleaning properties of many plant surfaces in 1975 and their transfer into biomimetic technical surfaces since 1992 resulted in a paradigm shift in surface technologies. A short survey of the state of the art is presented. The remarkable physical effect is based on at least a hydrophobic surface layer and a complex geometry of combined micro- and nanostructures on different length scales. Such kind of roughness minimizes the contact area between water and surface. Water forms droplets that virtually show no adhesion and roll off at lowest inclinations. In addition droplets pick up particulate contaminations and consequently clean the surface. Super-hydrophobic and self-cleaning surfaces are of great interest for many applications. First products (facade paints) are available on the market under the trade mark Lotus-Effect® since 1999, further products followed. This is one of the economically most successful results of biomimetics.

3.4.3. Anti-adhesive coatings: Why do elaborate insect attachment devices fail on some plant surfaces?

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Many aspects of insect-plant relationships deal with mechanical and chemical surface interactions. During the long period of co-evolution between flowering plants and insects, plants have developed not only structures attracting insects, but also a wide variety of surface attributes related to defence against herbivores. Various functions such as pollination, prevention of access to flowers, and insect trapping may involve similar general mechanisms related to surface structure and properties. Experimental data show that smooth, hairy, and felt-like substrates only slightly affect insect attachment. However, insects could not attach properly to surfaces covered with wax crystals. To explain anti-adhesive properties of substrata structured with crystalline waxes, four hypotheses are proposed: (1) Roughness-hypothesis; (2) Contamination-hypothesis; (3) Wax-dissolving-hypothesis; (4) Fluid-absorption-hypothesis. Our experiments on insect attachment to structured artificial substrates provide some evidences to the above hypotheses and inspirations for design of technological surfaces with anti-adhesive properties.

3.4.4. Biomimetics and water transport in plants: Putting negative pressures to work

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Water flow in the xylem is of technical interest due to the following properties: 1) An energy-consuming pumping mechanism is not required because water flow is generated by water loss at the transpiring sites, 2) water flow rates correspond to demand and 3) negative pressures can occur within the water column. Water transport in plants thus represents a unique biological transport system. There are currently no technical devices which make use of this principle. Devising technical systems using the principles of plant water transport have to be tightly coupled to the detailed analysis of the underlying biophysical mechanisms. There is, for example, evidence that fine structural adaptations of xylem conduits are involved in special interfacial effects which stabilize negative pressure states within the water column. In this contribution it will be demonstrated and discussed how technical applications for different uses can be realized by integrating the underlying biophysical processes of plant water transport.

3.4.5. The banana leaf as a teacher for lightweight design

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Biological structures consist of mechanical load carriers, which are highly optimized in terms of mechanical strength and minimum weight. A lot of parts of these structures act as tension ropes, even if this cannot always be identified immediately. The advantage of tension-loaded components is that they cannot fail by kinking or buckling like pressure-loaded ones, if their aspect ratio is too high. The non-woody banana leaf consists of non-stiff materials. However, by tricky arrangements of ropes and shell elements, structural stiffness of the stalk of the banana leaf results. Mechanical applications of the banana trick reveal a rope-dominated ultra-lightweight design, which is different from normal steel structures, because their unstable failure modes are avoided. Mechanical designs according to the banana leaf do not exhibit any instabilities and failures by predictable bending modes under overload. Although an absolute minimum of material is used, the structures were found to have an extreme high stiffness in prototypes.

3.4.6. Pressure, stabilisation, nature and Tensairity®

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The green tissue of plants is supported by the cellular turgor pressure. This pressure induced stability is crucial for the adaptability of the young plant. Growing older, many plants change their structural integrity by lignification. The transformation is very

energy expensive and thus only attractive for long living plants. In technology, load bearing structures are made of stiff materials as steel. Adaptability is hard to realize with these girders. It can be achieved with the light and deployable pneumatic structures. However, the load bearing capacity of these pressure stabilised structures is often too limited for e.g. temporary buildings. The technological gap between flexibility and strength, between transience and permanence was recently closed by the new structural concept Tensairity®, a development of Airlight Ltd in collaboration with prospective concepts ag. The synergetic combination of an airbeam with struts and cables leads to strong and adaptive Tensairity®-girders with minimal weight. Ideal applications range from temporary shelters and bridges to permanent wide span roof structures.

3.4.7. Self-repairing membranes for pneumatic structures: transferring nature's solutions into technical applications

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Over the last few years, plants have proved to be a real treasure trove as models for the construction of biomimetic technical structures and materials. One on-going project deals with the construction of technical membranes with rapid self-repair mechanisms inspired by plant structures. For analytically describing the (fast) self-repair characteristics of the parenchyma, lianas (e.g. *Aristolochia*) and herbaceous plants (e.g. *Phaseolus*, *Ricinus*) are used as model organisms. These plants react to fissures in their peripheral tissues with repair mechanisms on at least three hierarchical levels, which seal the lesion very effectively and secure the functional integrity of the plant structure. The mechanism on one level is based on fast strain-triggered deformation processes of pressurized parenchyma cells sealing the fissure. In cooperation with the Swiss company 'prospective concepts ag', biomimetically inspired self-repair functions are transferred into technical membranes of ultra-light pressurized beams based on the Tensairity® concept.

3.5.1. Mapping plant microtubules: the role of associated proteins in organizing MT arrays

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The spatial information for separating chromosomes and for the placement of cell walls is embodied in microtubule-based arrays that assemble and disassemble at different stages of the cell cycle. In higher plants there are four such assemblies and the way that such different structures can be organized from common building blocks depends largely on microtubule-associated proteins (MAPs) that regulate the dynamic and associative properties of microtubules. Many plant MAPs have been identified by homology with proteins whose functions are known in other eukaryotes but to target MAPs unique to plants we developed a proteomics approach for identifying proteins binding to Arabidopsis microtubules *in vitro*. We will describe progress in characterizing two novel MAPs, in particular MAP70, which is comprised of five isoforms. In addition, MAP65 is now recognized as the major cross-linking plant MAP and we will discuss recent work on how MAP65 unites microtubules in Arabidopsis cells and during secondary wall formation in the *Zinnia* model system.

3.5.2. Functional characterization of *Arabidopsis thaliana* gamma-tubulin complex components

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Plant microtubule arrays are involved in specific intracellular trafficking. The variety of microtubule arrays is related to dispersed assembly sites, but the nucleation process seems conserved in eukaryotes, as orthologues of genes expressing γ -tubulin Complex Proteins (AtGCP1 to 6) have been identified in the *A. thaliana* genome. Our aim is to functionally characterize AtGCP1 to 6. We have shown that AtGCP1, 2 and 3 interact with each other *in vitro* and are part of protein complexes *in vivo*. Using eGFP-tagging and immunolocalization in tobacco BY-2 cells, we have localized AtGCP1, 2 and 3 at the nuclear envelope, a well-characterized plant microtubule organizing centre. AtGCP2 and 3

domains which could be involved in targetting the entire proteins, and possibly nucleation complexes, to the nuclear surface have been identified. Recruitment and activation of gamma-tubulin nucleation complexes under cell cycle control could be instrumental in the regulation of microtubule nucleation in plant cells.

3.5.3. Ancient tubulin FtsZ in chloroplast and cell division

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Bacteria divide with the help of the filament-forming protein FtsZ. Based on biochemical as well as structural evidence this protein is regarded as the evolutionary ancestor of the eukaryotic tubulin. Nevertheless, plants harbour several *ftsZ* homologues in their nuclear genomes. Most of the encoded proteins are imported into plastids where they mediate chloroplast division. Additionally, FtsZ proteins can form filamentous networks in plastids for which we coined the term "plastosome", a dynamic structure which may help plastids to maintain and to change their shape. Moreover, some of these proteins are dual targeted to plastids and to the cytoplasm where they play, at least in the moss *Physcomitrella patens*, a role in cell division. Thus, they may serve as a molecular link between cell and organelle division. In vivo interactions of different FtsZ isoforms were demonstrated via FRET techniques. Isolation of FtsZ-interacting proteins in *Physcomitrella* is in progress. Financial support by DFG is gratefully acknowledged.

3.5.4. Cytokinesis in the view of the microtubule-associated protein PLE/MAP65-3

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In plants proper execution of cytokinesis is guided by a plant specific cytoskeletal structure, called phragmoplast, consisting of microtubules (MT) and actin filaments. In the *Arabidopsis thaliana* cytokinesis defective mutant *pleiade* (*ple*) this structure is impaired. The spotty expression pattern in root meristems of *PLE/MAP65-3* (microtubule associated protein 65-3) and the localization of *PLE/MAP65-3* to mitotic MTs, in particular to the phragmoplast midzone in *Arabidopsis thaliana* cell suspension cells is already an indication that *PLE/MAP65-3* is regulated in a cell cycle-dependent manner. One post-translational regulation might be governed by cyclin-dependent kinases (CDK) and / or mitogen-activated kinases (MAPK), since *in silico* analysis reveals several putative MAPK and CDK phosphorylation sites. The characterization of these sites will be presented. This work is supported by the Austrian Science Fund (FWF P16410-B12)

3.5.5. TMV movement and the plant cytoskeleton

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Studies in higher plants have revealed the existence of RNA species that travel cell-to-cell and through the vasculature to serve as signaling molecules in plant development and gene silencing. RNA viruses encode movement proteins (MP) that may interact with the RNA transport machinery to spread the viral genome from cell-to-cell. The MP of Tobacco mosaic virus (TMV) is believed to form a ribonucleoprotein complex (RNP) with viral RNA (vRNA) and to represent the core of the infectious particle that spreads between cells. Previous studies indicated that the movement process is correlated with the ability of the MP to interact with microtubules. Here, it is reported that in cells at the spreading front of infection, the MP:GFP localizes to distinct mobile particles some of which move along filaments, presumably microtubules. Similar particles are also observed if viral RNA is labeled with GFP, suggesting that they may represent the proposed vRNPs.

3.5.6. KAT1 and KAT2 kinesin like genes are involved in the spatial control of cytokinesis in *Arabidopsis thaliana*

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Mitotic microtubule assemblies (preprophaseband, spindle, phragmoplast) are involved in the spatial and temporal establishment of the cross wall between daughter cells. The *Tan1* (*Tan1*) gene is required for the proper orientation of these microtubule (MT) arrays during mitosis in maize. Maize orthologs of *Arabidopsis* (*At.h.*) kinesins KAT1 and KAT2 were identified as interaction partners of TAN1 in a yeast two hybrid screen; KAT1 showed interaction with ATN (*At.h.* TAN1 like) in the yeast two hybrid experiment. Expression patterns of ATN, KAT1 and KAT2 are overlapping in immature flower buds and root tips, both of which are enriched in dividing cells. In a reverse genetic approach, we identified multiple *kat1* and *kat2* mutant plants, who do not exhibit obvious alterations from the wild type phenotype; the double mutant *kat1;2* plant develops into a semi dwarf. In the *kat1;2* double mutant, defects occur already early in embryogenesis giving rise to misplaced cell walls and consequently miss-shaped embryos and adult plants; in *kat1;2* mitotic microtubule arrays are often tilted from the predicted orientation, as reflected in the oblique position of the new cell wall.

3.5.7. Shape and photosynthesis-dependent subcellular distribution of mitochondria in characean internodal cells depend on simultaneous interaction with actin filaments and microtubules

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Shape, size, number and distribution of mitochondria in characean internodes varied according to the growth status and the metabolic activity within the cell. Vermiform or amoeba-like mitochondria were present in elongating internodes whereas younger and older cells contained short, rod-like organelles. Mitochondria were evenly distributed and passively transported in the streaming endoplasm. In the cortex, mitochondria were sandwiched between the plasma membrane and the stationary chloroplasts and distributed in relation to the pattern of pH banding. Highest mitochondrial densities were found at the acid, photosynthetically more active regions. In elongating cells, the shape and motility of mitochondria depended on the simultaneous interaction with both actin filaments and microtubules suggesting that the mitochondria possess the appropriate motor proteins which can be used either as tracks for migration or for immobilization. The cortical cytoskeleton probably controls the spatio-temporal distribution of mitochondria and promotes their association with chloroplasts, necessary for exchange of metabolites and detoxification.

3.5.8. Dynein-dependent microtubule motility polarizes the tubulin cytoskeleton in the plant pathogenic fungus *Ustilago maydis*

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In the plant pathogen *Ustilago maydis* interphase microtubules (MTs) are nucleated at polar MTOC that send the plus-ends towards the cell poles. Interestingly, assembled MTs undergo rapid motility. Such motility participates in MT polarization in axons, but its role in *U. maydis* is unknown. Here we show that dynein mediates MT motility and that its activity is essential for organizing the interphase array. After disruption of MTs by benomyl and subsequent washout of the drug, MTs reappeared at numerous sites within the cytoplasm. Tagging the MT plus-ends with an fluorescent EB1-homologue revealed that these MTs had an anti-polar orientation. However, the MT array polarized within less than 2 minutes in a dynein-dependent fashion. Furthermore, no MT motility was seen in dynein mutants and MT polarization is rapidly lost at restrictive conditions. These data suggest that dynein-based MT transport participates in polarization of the tubulin array in interphase.

3.6.1. Ancient DNA and the history of maize in North and Middle America

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Archaeology and genetics have to varying degrees independently shed light on the process of maize domestication. Archaeologists are limited to interpreting the phenotypic characteristics of archaeological cobs, while geneticists interpret the genetic consequences of human and natural selection by analyzing modern maize and its wild ancestor teosinte. Recent innovative approaches in the study of ancient plant genetics create the possibility of a more comprehensive understanding of the domestication process. Our research shows how ancient DNA can be used to address several different sets of questions concerning the domestication of maize. First, how the plant architecture or biochemical properties of starch, characteristics selected by maize farmers, but not directly observable in the archaeological cobs can be determined. Our analysis of three selected genes affecting maize architecture as well as starch and protein properties, show that all three characteristics were present in Mexican maize 4,400 years ago. For one of the genes we observe a closer relationship of the inbred maize Northern Flint from Northeastern North America to ancient and modern Southwest maize than to ancient and modern Mexican maize.

3.6.2. Desiccated plant remains and archaeogenetics

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Desiccated plant remains often display excellent preservation of biomolecules, enabling DNA sequences to be obtained with good success rates from material up to 2000 years in age. This means that, when such material is available, ancient DNA can answer questions relating to the biogeography of cultivated plants. For example, through use of cobs obtained from cave sites, it has been possible to study the origins of South American maize, without the complicating factors introduced by the movement of genotypes during the post-Columbian period. This work has led to a model in which the two Central American agricultural systems - highland and lowland -- generated separate expansions of maize cultivation into South America. One expansion centred on a highland culture that spread through the Panama highlands into the Andean regions on the east of South America, and the second expansion centred on a lowland culture which spread along the lowlands of the northeast coast of South America, entering the continent through the river systems. Comparison between ancient allele sequences also shows that maize has undergone a rapid rate of evolution since domestication.

3.6.3. The survival of DNA in charred seeds and its use in the study of plant population genetics

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The presence of ancient DNA in charred plant remains has been reported by several groups although the validity of these results has been questioned. However recent research has confirmed the survival of DNA in wheat seeds charred for several hours. This study was aimed at improving the sensitivity of plant DNA detection methods by optimising DNA extraction and target amplification from artificially charred wheat seeds. Nuclear and cytoplasmic DNA amplification targets were assessed for their ability to survive the charring process. Charring regimes were chosen that result in the seed morphology that most closely resembles that found in archaeological material. The results have proven that DNA survival in charred seeds is remarkable and the new highly sensitive techniques will now be applied to genuine archaeological samples. The results will form part of a larger project aimed at using the genetic variation present in cereal landraces to help trace the trajectories by which agriculture spread into Europe.

3.6.4. Ancient olive DNA from pits: preservation, amplification, and sequence analysis

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Olive (*Olea europaea*) was domesticated by vegetative propagation of selected wild individuals with superior fruit. Later new cultivars were established repeatedly from feral trees or from crosses between wild and domesticated trees. Thus, the genetic background of modern domesticated lines is a mixture of ancient cultivars and local wild trees. Information coming from fossil DNA may illuminate the process of olive domestication because such DNA sequences provide data about genomes closer to the original wild population. To do this a reliable source for fossil DNA must be established, but the hot and humid regions of olive cultivation are not favorable to DNA preservation. In order to find well preserved and authentic fossil DNA, we defined chemical parameters of pits by IR spectroscopy that correlate with parameters of DNA quality such as template length in an olive-specific repeat array, and template quantity as determined by real time PCR amplification. Pits that past these tests contained high quality fossil DNA that we used for phylogenetic analysis. We present the first ancient olive DNA sequences and compare them to modern wild, feral and domesticated lines.

3.6.5. New approaches for obtaining plant DNA from museum collections

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Herbaria contain vast quantities of plant material, and the ability to obtain usable DNA from these collections has the potential to augment many research projects. We report results from experiments on the efficacy of protocols modified for museum material, as well as attempts to obtain DNA from different plant organs, including seeds, stems, and flowers. One of the major modifications to our protocol was to proceed with the extraction without maceration of the tissue. With this non-destructive approach we have obtained DNA from plant tissue up to 200 years old. This approach has also worked on a variety of plant material preserved in different ways. For example, DNA was extracted from orchid pollinia that were attached to museum collections of bees. This was accomplished without destroying the pollinia or the bees, and allowed for further study of both organisms. As technical challenges are overcome, the utility of plant DNA from museum collections will continue to grow, and the applications of these results to ongoing research projects will be emphasized.

3.6.6. Ancient wine - tracing origins by microsatellite analysis of archaeological *Vitis vinifera* seeds

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In three out of six archaeological samples of *Vitis vinifera* seeds from sites in France and Hungary with evidence of wine-making, microsatellite markers VV2S, ZAG21 and ZAG62 were successfully PCR amplified. Through comparison with databases of modern *Vitis* cultivars, the *Vitis vinifera* sample from the Roman site Aquincum Kaszásdűlő (2nd -4th cent. AD) was assigned with high probability to Italian and Croatian populations based on three microsatellites. In the two samples from France two microsatellites were amplified, which were sufficient to narrow down the regional provenance: seeds from a Greek city at Marseille (5th cent. BC) were assigned to either Austria/Germany or France, seeds from an Iron Age site at Gailhan (5th cent AD) originated with higher probability from France than from either Austria/Germany or Italy. These preliminary data show that microsatellite sequences of archaeological *Vitis vinifera* seeds can be used to investigate the origin and history of ancient cultivars

3.6.7. Nuclear gene sequences and DNA variation of *Cryptomeria japonica* samples from the postglacial period

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Genomic DNA was extracted from heartwood blocks of six *Cryptomeria japonica* individuals that had been buried for about

3600 years. Attempts were made to determine the sequences of five nuclear genes following PCR, using expressed sequence tag (EST) markers. We detected 15 nucleotide substitutions and four indels in a partial GapC gene sequence among 13 individuals of the buried and an extant population. For the entire haplotypes of the GapC region, pi and theta were 0.0063 and 0.0010, respectively. Estimates of DNA divergence statistics suggest that differentiation between the two populations was not great. However, permutation tests gave FST and KST values rejecting the null hypothesis (that populations were not differentiated). The significant level of haplotype diversity in the extant population compared to the buried population might be the result of gene flow from neighbouring artificial forests. Alternatively, it is possible that we failed to detect all the DNA variation in the buried population because of clonal growth and/or genetic structure in the buried population.

3.7.1. Are fungi living in lichens and plants the missing link to our understanding of fungal and plant evolution?

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The discovery of an unexpectedly high diversity and abundance of cryptic fungi in healthy leaves of plants and thalli of lichens begs an explanation for their role as putative symbionts with regard to their respective hosts and ecosystems. Did the diversification of lichens and land plants lead to the diversification of endolichenic and endophytic fungi by providing new substrates in new environments for enhanced rates of fungal speciation? Or were beneficial interactions of these fungi with their hosts conducive to a higher rate of speciation among lichen-forming fungi and plant lineages? Do endophytes and endolichenic fungi serve as a reservoir for the evolution of fungal pathogens? Using a phylogenetic framework for the Ascomycota, the times of origin and diversification of fungal endophytes, endolichenic fungi, lichen-forming fungi, and of a selection of fungal pathogens will be estimated and compared to divergence times of main lineages of land plants.

3.7.2. Evolution, host specificity and ecology of sclerotial basidiomycetes that associate with lichens

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The vast majority of described lichenicolous fungi are ascomycetes, but lichenicolous basidiomycetes are also known. The ones that have the widest host amplitude also produce conspicuous sclerotia or bulbils, structures thought to have a dormancy or dispersal function. The best known sclerotial lichenicolous fungus is the virulent pathogen, *Athelia arachnoidea*, the only member of the "athelioid" clade of basidiomycetes that is both lichenicolous and sclerotial. There is also a single described sclerotial lichenicolous species in the genus *Leucogyrophana*, a genetically heterogeneous group in the "bolete" clade. The bulbilliferous genus *Marchandiomyces* in the "corticoid" clade includes both lichenicolous and lignicolous species. Another group of bulbilliferous basidiomycetes that are at least facultatively lichenicolous is in the "cantharelloid" clade, and includes species in the genus *Burgoa*, all apparent anamorphs of *Sistotrema*. Phylogenetic analysis of rDNA sequences indicates that in all of these groups, the lichenicolous habit has evolved independently several times, probably from nonlichenicolous ancestors.

3.7.3. Lichens as a habitat for fungi - a molecular survey

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The presented study aims to assess the distribution and host/substrate selectivity of lichen-associated fungi, and to screen the habitat lichen for undiscovered species and new phylogenetic groups. 1086 fungal strains were isolated from lichens of the *Letharietum vulpinae*. They were pre-sorted according to culture characteristics, and subsequently grouped by ITS/5.8S nrDNA sequence data. The 18S nrDNA was sequenced for representatives for each ITS genotype. The sequences were aligned with all publicly available high quality SSU nrDNA sequence data of Ascomycetes. Phylogenetic analyses of the alignment revealed the majority (91%) of the ITS genotypes to

cluster the Dothideomycetes (35%), Chaetothryiomycetes (20%), Leotiomyces (17%), Sordariomycetes (11%), and Lecanoromycetes (8%). Group specific oligonucleotide probes targeting the rRNA were designed for each of the 23 clades containing ITS genotypes of the isolated fungi, and immobilized on epoxy glass slides. These microarray chips were hybridized with fluorescent labelled RNA isolates of various lichens.

3.7.4. Endolichenic fungi: random inhabitants or symbiotic partners

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Studies of lichenicolous fungi (secondary fungi associated with lichen thalli) have been restricted, almost exclusively, to fungal species with visible reproductive structures on lichen surfaces. However, the potential for fungi to occur asymptotically within thalli (e.g., as endolichenic fungi, analogous to endophytes of plants) remains mostly unexplored. We used a gradient of surface-sterilization to examine fungal communities associated with asymptomatic thalli of foliose lichens (*Lobaria*, *Nephroma*, *Peltigera*, and *Umbilicaria*) representing various habitats in neotropical, temperate, boreal, and arctic sites. Based on the sequences of the entire ITS region obtained from all cultivable fungal isolates, we explored diversity, taxon composition, geographic structure and host specificity of endolichenic fungi. Phylogenetic placement and affiliation of selected endolichenic fungi, endophytes isolated from different plant lineages, and representatives of major lineages of Ascomycota were explored using nuclear ribosomal genes, and used as the basis for a discussion of the evolution of the endophytic and endolichenic habits.

3.7.5. Inferring symbiont evolution across terrestrial plant lineages: Endophytic fungi from the tropics to the tundra

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Fungal endophytes are ubiquitous among green plants. Their potential to have coevolved with host plant lineages, to have descended from pathogenic or saprophytic fungi, and to inform our understanding of the fungal tree of life, have not been fully explored. We surveyed endophytes inhabiting photosynthetic tissues of all major plant lineages at sites from the neotropics to the Arctic. Over 6400 isolates were recovered from bryophytes, ferns and allies, conifers and angiosperms. NuclITS data were obtained for 2030 isolates to assess endophyte diversity, host affinity, and spatial structure, and to select taxa for multilocus phylogenetic analyses. We recovered high upper-level diversity of endophytes at higher latitudes, whereas temperate and tropical sites were characterized by high species-level diversity. Many endophytes have wide distributions, but several clades showed strong host affinity and local distributions. Abundance of major fungal clades differed among host lineages within sites, and among related hosts at different sites. The impact of these cryptic fungi on hypotheses regarding the evolution of plant-fungal symbioses will be discussed.

3.7.6. Phylogeny of endophytic Xylariaceae from multiple photosynthetic hosts

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The Xylariales are regularly identified as dominant components of the endophyte flora from phylogenetically and geographically disparate hosts. Using sequence similarity analyses of the internal transcribed spacer of nrDNA (ITS) from Xylarialean endophytes of liverworts, mosses, ferns, gymnosperms, angiosperms, and lichens, as well as non-endophytic Xylarialean species, we show that certain Xylarialean endophyte sequence groups are globally distributed, non host-specific, and show low sequence similarity to non-endophytic species. Our data further suggest that other

endophyte groups have geographically local distributions and/or are more closely allied with non-endophyte taxa. Results of multi-locus phylogenetic analyses (nuclear ribosomal LSU, SSU, and 5.8S) place Xylariales endophytes in the Amphisphaeriaceae and Xylariaceae, and further imply that the endophytic habit occurs in multiple lineages within the Xylariaceae. Well-supported clades are identified within the genus *Xylaria*, and there is low sequence divergence in LSU, SSU, and 5.8S among these groups.

3.7.7. Conflicting forces in asexual grass endophyte symbiosis

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Endophytic fungi are considered to be mutualists because they receive nutrition and protection from the host plant, and in grasses also asexual dispersion by growing into the host's seeds, while the host receives benefits through increased resistance to herbivores, pathogens and drought and flooding stress, and enhanced competitive abilities. However, benefits to the partners are only rarely symmetric and conflicting selection forces are likely to destabilize them. For example, (1) the asymmetry in dependence, (2) conflicts between host and fungal reproduction, (3) genetic mismatch between the partners, and (4) energetic costs of harboring the endophytic fungus might destabilize the symbioses. Because of these conflicts, life histories of grass endophytes are likely to be dynamic. The question is how ecology and genetics interact to shift fungal life history traits between the extremes of sexuality and asexuality and pathogeneity and mutualism.

3.8.1. Floras, the life-blood of plant taxonomy

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Consideration will be given to the importance of local, national, regional and global Floras in the modern age. At a time when taxonomy is taught less and less in universities and funding is directed increasingly to other branches of botany, we should not forget that the ability to correctly identify the plants on which we work is still of fundamental importance. In particular, conservation issues demand more and more knowledge of plants in the field, but it is known that our basic knowledge of the taxonomy of threatened plants is often very poor. Lip-service is often paid to the need for taxonomic work, but support for it is declining. The role of individuals, teaching establishments, botanical institutions and international bodies will be discussed. Floras have been the great stimulus for taxonomic work for two centuries, and offer the possibility for a focus of attention to attract the much-needed funding which will be essential if we are ever to complete the inventory of plant diversity. The main issue is not whether Floras should be produced in hard copy or in electronic form; the great need is to get the taxonomy done in the first place.

3.8.2. Different scales - different constraints (or not?)

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Some people might think that the considerations and constraints encountered in producing a modern Flora treatment would be the same, whether the region being covered was big or small. Others would think that this would certainly not be true. What is the reality? We will explore this question by comparing current Floras covering various regions of different sizes, notably the recently completed 'Flora of New South Wales' (covering a medium-sized Australian State, with c. 6 000 vascular species), the continuing 'Flora of Australia' (for an island nation/continent with c. 20 000 species) and the continuing IOPI Species Plantarum Project's 'Flora of the World' (with 250 000-300 000+ species, depending on which estimate of global numbers one follows). All three are available in hard-copy and are currently being made available electronically. We will look at the features of both hard-copy and electronic versions.

3.8.3. Floras & informatics. A new age for floristic data

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Recently, some informatic applications have been developed to help producing morphological descriptions, etc. A demonstration of a new programme will be done. This programme has as characteristics: - it works over the Internet - can be feed from traditional descriptions, even already published - for any one of the species it displays a dot map made on-the-fly, a picture, a plate, a description, the list of synonyms, an updated list of localities, etc. - it shows geographic, cytological or graphical information taken from both sources: bibliography and herbarium specimens - it avoids the problem caused by the synonyms -the information related with a single species can be found under more than a single name- through a routine developed to understand the nomenclature and synonymy - it assist as well for specific identification without using dichotomical keys, but a interactive way of searching - it produces a list of any of the characters used for each taxon in a dichotomical identification key As summary, we can show a system to help providing taxonomic and floristic information not as a printed flora, but in a modern and interactive format through the Web.

3.8.4. California as a case study in the evolution of floristic modeling

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Although floristics is largely perceived as an exercise in information compilation and management, it is more accurately understand as a massive, on-going, internationally collaborative effort to model biodiversity. The taxonomic components (e.g., species) are not free-standing static entities, but rather dynamic hypotheses that derive their identities from the larger floristic framework in which they are nested. California provides an excellent case study of this concept and the various stages of floristic evolution. Initial cataloguing led to increasingly sophisticated (and competing) floristic frameworks that incorporated the latest ecological, biogeographic, biosystematic, and phylogenetic developments, with significant pioneering efforts occurring in California. The electronic era now allows an improved expression of the dynamic nature of floristic frameworks, as reflected by current developments in California floristics (i.e., the Jepson On-Line Interchange, ucjeps.berkeley.edu/interchange.html).

3.8.5. Diversity of approaches and layouts in regional "Floras" in Russia and neighboring countries

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"Flora of the USSR" (1934-1964) was followed by "Floras" of all former Soviet republics (except Russia) and several flora projects on the large regions of the former USSR, which have been accomplished (European part, Siberia, Far East, Middle Asia) or are near finishing (Caucasus). Regional and republican "Floras" generally followed the scheme of the "Flora of the USSR", but there are several variations in approaches and layouts between "Floras" and even different treatment in the same "Flora". V. L. Komarov species concept adopted in the "Flora of the USSR" can not be regarded as simply "extreme splitting" but reflects several natural features of the territory of study (vast lowland areas). A peculiar character of "Russian" Floras is a tradition to use schemes of more or less natural regions to describe geographical distribution; "Floras" of Siberia and the Far East have distribution maps for almost all species. At the same time there are no specimens lists in the majority of series. A concept of the new "Flora of Russia" is now in preparation. This "Flora" has a good chance to be a synthesis of advantages of both Russian and West European taxonomic schools.

3.8.6. Flora-writing exemplified by classical, conservational and unconventional models

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There are many ways to compile a Flora. The *Flora of Turkey* is a remarkable 10-volume work completed within a quarter-century of its inception. A review of the factors contributing to the Flora's success is provided. The *Flora of Turkey* is a classical Flora with an additional supplement recently produced by Turkish botanists. *Flora Hellenica* is another example of a classical Flora. Some aspects of its background and production are briefly mentioned.

Conservationists consider the endemic flora of a country needs protection for all time, and an opportunity arose to prepare a three-volume work on the endemic flora of Greece. The first volume, covering the Peloponnese, was published in 2001. Flora contributions and monographic work both have their uses. An unconventional model of Flora-writing is presented after the revision of a large family of plants. The Brassicaceae is a diverse group, several members of which have great economic importance. Biodiversity in the family is unquestionably demonstrated, together with comments on relationships. In summary, a good Flora survives long after the flora has gone.

3.8.7. Managing data for floristics

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Databases can hold all the data required for the writing of a Flora, and can do so with much improved accuracy and precision, if data acquisition is managed carefully. Floristic data can then also readily be distributed and shared. All floristic data depends on the nomenclature. Data on literature and specimens (both with images), people, geography and distribution follow, and after that many other data types such as descriptive characters, keys, maps, uses and ecology. A draft of a Flora can be generated directly from the database, by selecting the data types and output formats. Standards are vital, as for authorities, citations and geography, and in standard data formats. Data retrieval is then more reliable, and data sharing easier. High quality can be achieved by various means. First, checks should be made when data is first entered. Later, special software can verify such as the dates for a certain person, and their publications and collections. Other checks can ensure that all relevant data is present, and at the planned level of detail. Examples will be given from the Flora of Turkey, the Euro+Med, the Species Plantarum Flora and the world Rosaceae databases.

3.9.1. Phylogeny of the Lamiales, with special reference to family Plantaginaceae, sensu APG

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The clade Lamiales was first recognized on the basis of rbcL phylogenies of flowering plants and comprises the orders Lamiales, Scrophulariales, Plantaginales of previous authority-based classifications and taxa from related groups. The traditional family Scrophulariaceae was recognized to be a polyphyletic assemblage consisting of several clades now accepted as distinct families in the recent Angiosperm Phylogeny Group (APG) classification of angiosperms, including Scrophulariaceae s.s., Orobanchaceae, Calceolariaceae, Stilbaceae p.p., Phrymaceae, and Plantaginaceae. Perhaps the most widely distributed and biologically diverse segregate clade is the Veronicaceae (family Plantaginaceae) with close to 100 genera and 2000 species. Cosmopolitan in distribution, this group includes members that have been assigned to several distinct families on the basis of their diverse life histories and reproductive biology, including aquatic plants, wind and water pollinated plants, and plants with dramatically reduced floral structures. Plantaginaceae itself has strong support from plastid DNA data, but its position within Lamiales is unclear.

3.9.2. Phylogeny and biogeography of Cheloneae

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Cheloneae, as originally circumscribed, included 26 genera of Scrophulariaceae with cymose inflorescences. The number of genera included in the tribe, and the taxonomy of Scrophulariaceae has changed dramatically over the past decade as more sophisticated morphological and molecular analyses have been done. A phylogenetic study using ITS and *matK* sequence data was conducted on tribe Cheloneae to test for monophyly, examine biogeographic patterns, and assess relationships. The results of this study provide evidence that *Collinsia* and *Tonella* should be transferred from Collinsieae to Cheloneae and that Russelieae should be expanded to include *Tetranema*. Our results also indicate a New World origin for Cheloneae with dispersal of the Asian component over Beringia. North American Cheloneae may have originated in the Klamath Region of the western United

States with expansion to the Rocky Mountain/Columbia Plateau region followed by diversification of several genera. We propose a post-Tertiary evolutionary radiation of the large North American genus *Penstemon*.

3.9.3. Phylogenetic investigations of the Antirrhineae: Implications for taxonomy, biogeography, and character evolution

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The monophyly of the Antirrhineae lineage is supported consistently in molecular phylogenetic analyses, which is concordant with unique morphological (e.g., pericidal capsule dehiscence) and iridoid (e.g., antirrhinoside) characters, and its near uniform delimitation as a tribe or subfamily within the historical 'Scrophulariaceae'. The primary unresolved systematic hypotheses involve sister group and infra-lineage relationships, generic boundaries, and patterns of character evolution. Analyses of plastid and nuclear molecular markers have identified several clades within the Antirrhineae such as 'Maurandiyinae' + *Asarina* + *Cymbalaria*, *Anarrhinum* + *Kickxia*, and *Antirrhinum* s. l. + *Mohavea* + *Chaenorhinum*. Our investigations focus on resolving phylogenetic and biogeographic relationships among the Old World/New World 'Maurandya' clade, North American/South American taxa *Gambelia* + *Galvezia*, and Old World/New World species of *Linaria* + *Nuttallanthus*. Biogeographic patterns and the evolution of morphological and phytochemical characters, chromosome numbers, and cross incompatibility are examined within several lineages.

3.9.4. Searching for morphological synapomorphies for the clades in the Veronicaceae

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Veroniceae have arisen as a surprising sister group to *Plantago* (plus *Aragoa*) in DNA sequence analyses. A new infratribal and infrageneric rearrangement for the *Veroniceae* and for *Veronica* has been proposed based on the most recent evidence from DNA sequence data (three DNA regions, both nuclear and plastid), morphological evidence, and biogeographical considerations. Looking for morphological synapomorphies is apparently misleading, especially for each subgenus in *Veronica* s. l. (including *Hebe*, *Pseudolysimachion*, *Synthyris*) due to difficulties arising from widespread homoplasy (mainly parallel evolution). In an attempt to overcome these difficulties, several previously under-explored morphological characters have been studied. A combined analysis of some of them (e. g. base chromosome number, seed coat) shows that, although this is not an easy task, it is also not a misconceived plan at least for some clades. Information on character evolution in *Veroniceae* is important to determine possible synapomorphies for the *Veroniceae-Plantago-Aragoa* clade.

3.9.5. Biogeography of Plantaginaceae: Family-level trends and intriguing generic patterns

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Recent molecular phylogenetic work on Plantaginaceae s.l. has revealed new insights into the evolution and biogeography of the family as a whole, as well as some of its tribes and genera. Plantaginaceae consists of two main sister lineages: 1) a mostly New World clade (*Angelonia* and relatives plus some members of Gratiroleae), and 2) a mostly Old World clade (*Veronica*, *Antirrhinum*, etc.) plus its sister clade of New World Cheloneae, *Russelia* and *Tetranema*. An overview of different biogeographic hypotheses will be discussed for Plantaginaceae, with an emphasis on shifts between the Old and New Worlds. In addition, we will focus on the biogeography of *Ourisia*, *Digitalis* (including *Isoplexis*), *Globularia*, and other genera with existing phylogenies. Finally, a synopsis of some of the remaining genera in the mostly New World clade will be provided, highlighting potentially interesting biogeographic distributions and underscoring the need for more phylogenetic research on these groups.

3.9.6. Phytochemistry of Plantaginaceae

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Plantaginaceae have been much investigated with regard to its chemical constituents. Iridoids and phenylethanoid glycosides are widespread in the family and show a suitable variation to be useful as taxonomic markers. The iridoid glucosides aucubin and catalpol as well as their esters are, together with the phenylethanoid glycoside verbascoside, perhaps the ancestral constituents in the family. With some variations, these are present in most Plantagineae, Veroniceae, Globularieae and Cheloneae. On the other hand, Gratioleae and Antirrhineae mostly lack these compounds and the former instead contain triterpene glycosides, while the latter is characterized by the iridoid antiirrhinoside. The specialized aquatics *Callitriche* and *Hippuris* both contain the expected compounds, while the creepers *Ellisiophyllum* and *Sibthorpia* are almost completely depauperated in iridoids. Within Veroniceae, the genera *Lagotis*, *Wulfenia* and *Veronicastrum* are clearly different from *Picrorhiza* and *Veronica*. A preliminary investigation of the former genus *Hebe* shows that this taxon chemically fits well within *Veronica*.

3.9.7. Phylogenetic relationships within the genus *Linaria* Mill. (Plantaginaceae): the case of sect. *Supinae*

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Linaria is the largest genus in the tribe *Antirrhineae*, and it is distributed throughout the northern hemisphere. Its main centre of distribution is located in the Mediterranean basin. According to Sutton (1988), the genus is divided in five sections: *Linaria*, *Supinae*, *Pelisserianae*, *Versicolores* and *Diffusae*. Phylogenetic relationships among the sections were investigated using nuclear DNA (ITS: internal transcribed region) and plastid DNA (*trnL-F*: the *trnL* intron and the *trnL-trnF* intergenic spacer). Section *Supinae* includes many closely related taxa, many of them endemic or restricted to small areas. Both regions (ITS and *trnL-F*) showed a low level of nucleotide variability and were not informative at the specific level. Most of the taxa in the section formed a polytomy in the strict consensus tree. To resolve this problem, we also analysed many of the *Supinae* taxa using AFLP (amplified fragment length polymorphisms).

3.10.1. Ecological genetics of homoploid hybrid speciation

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New species may arise via hybridization and without a change in ploidy. This process, termed homoploid hybrid speciation, is theoretically difficult because it requires the development of reproductive barriers in sympatry or parapatry. Theory suggests that isolation may arise through rapid karyotypic change and/or ecological divergence of hybrid neospecies. Here, we evaluate ecology's role in hybrid speciation, with an emphasis on the underlying genetics. We review theoretical developments related to the ecology of hybrid speciation, propose a set of explicit questions that must be answered to verify the role of ecological selection, discuss published work with reference to these questions, and report new data supporting the importance of ecological selection in the origin of hybrid sunflower species. We show that that homoploid hybrid species are most likely to arise under conditions of strong ecological selection and that ecological divergence is critical for their persistence.

3.10.2. Mixing genomes: changes to genome structure and gene expression during diploid hybrid speciation in *Senecio*

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The detection of recently originated plant species provides significant opportunities for examining structural and functional genomic changes that occur during and immediately following plant speciation. The Oxford ragwort, *Senecio squalidus*, is a new diploid hybrid species that originated in the British Isles within the past 300 years from material introduced from a hybrid zone between two species, *S. aethnensis* and *S. chrysanthemifolius*, that

occur on Mt Etna, Sicily. We have generated linkage maps of all three taxa using AFLP, SSR and allozyme markers to determine the genomic contributions of the two parent species to *S. squalidus*, and to examine possible alterations to the genome structure of the hybrid during or shortly following its origin. In addition, we shall report the results of a gene expression assay that has employed a recently developed anonymous cDNA microarray resource to examine changes to floral transcriptome during the origin of *S. squalidus*.

3.10.3. Swamping the swamp gum: Habitat fragmentation increases hybridization in a woodland eucalypt

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Habitat fragmentation can result in changes to population parameters that may promote interspecific hybridization. We examined the influence of absolute and relative population size on hybrid production in 16 populations of *Eucalyptus aggregata*, a tree species occupying woodland remnants in south eastern Australia. Proportion of hybrid offspring in open-pollinated families increased with declining absolute and relative population sizes, such that hybrid seed were 45% of seed crops in populations <40 trees. Growth data show hybrids to be vigorous, but chemical analysis of leaves indicate significant changes in the quantity and identity of insect defence chemicals. In terms of *in-situ* conservation, these results suggest that hybridisation may result in genetic assimilation (swamping) of *E. aggregata* by its common relatives in fragmented landscapes. For *ex-situ* conservation, seed crops from such sites should not be used for revegetation as they contain many hybrid genotypes.

3.10.4. Pollination strategies and natural hybridization in orchids

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Natural hybridization often occurs upon secondary contact between species that are not fully reproductively isolated. The evolutionary consequences depend on the strength of pre-zygotic and post-zygotic barriers among the parental taxa. Although orchids are typically thought to attract highly specific pollinators, hybridization has been widely reported in this group. In Mediterranean orchids, different levels of pollinator specificity exist. Food-deceptive *Orchis* and *Anacamptis* species show weak pollinator specificity, whereas the sexually deceptive *Ophrys* species display a highly specialized pollination strategy. Experimental evidence indicates that groups with high pollinator specificity largely rely on pre-mating reproductive barriers, whereas groups with weak pollinator specificity show strong evidence for intrinsic post-mating reproductive barriers. Molecular analyses of orchid hybrid zones support these results and indicate that the types and strength of reproductive barriers directly affect the rate and the evolutionary consequences of hybridization.

3.10.5. Hybrid zones as bridges to gene flow across species boundaries in *Silene latifolia* and *S. dioica*

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Silene latifolia and *S. dioica* (Caryophyllaceae) are closely related species that differ in habitat preferences and floral traits. *Silene latifolia* grows in relatively sunny and dry habitats and has white flowers that are pollinated predominantly by nocturnal moths, whereas *S. dioica* grows in nutrient-rich and moist habitats and has red flowers that are pollinated during the day by bees and butterflies. Where the habitats of both species meet, hybrid populations are formed. Hybrids are viable and fertile and may thus serve as bridges to gene-flow across species boundaries. Using molecular markers we characterize the genetic composition of two large hybrid zones in the Swiss Alps and investigate the extent of introgression from *S. latifolia* into *S. dioica* and vice versa using autosomal and sex-linked markers. The results provide new insights into the porosity of *Silene* genomes and form the basis for the study of adaptive trait introgression.

3.10.6. Towards the molecular genetic analysis of the barrier to gene flow between *Populus alba* and *P. tremula*, two ecologically divergent European forest trees

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Barriers to gene flow between ecologically divergent species are of special interest to students of adaptation because the genes conferring ecological differences are expected to segregate in hybrids. Hence, hybrid populations offer a powerful tool for the genetic analysis of adaptively important traits. Perhaps the biggest impediment to unlocking the potential of interspecific populations lies in the difficulty of generating multi-generation crosses in long-lived species, e.g., forest trees. Natural hybrid zones offer an attractive alternative: the increased genetic variability and admixture linkage disequilibrium (admixture LD) present in hybrid populations can be utilized for association studies. We have launched a research program to study the potential of admixture LD - based studies in hybrid zones of *Populus alba* and *P. tremula*, two ecologically divergent forest trees, and first results of this ongoing work will be presented.

3.10.7. Hybridization and polyploidy: crossing the ecological divide

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Speciation often is accompanied by the shifts in the ecological and temporal niches (new adaptations) of populations. The genetic resources required to adapt to new environments are best afforded by mixing disparate gene pools. Yet a rich genetic milieu is not a sufficient substrate for speciation, because recombination and gene flow from the progenitor may prevent the stabilization of novel genotypes (adaptations). Polyploidy coupled with hybridization is key to ecological divergence with its attendant new adaptations, because it congeals new genotypes and erects genetic barriers around them, and magnifies ecological disparities between hybrid derivatives and progenitors. Polyploidy coupled with hybridity also may enhance lineage persistence by elevating immediate fitness and evolutionary flexibility through genome-specific gene silencing, intergenomic recombination, genome reorganization, and increased genetic robustness.

3.11.1. Forests in a CO₂-rich world: Old questions, new challenges

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The increasing amount of CO₂ in the atmosphere is a global change that will influence forests for many decades into the future. Photosynthetic assimilation of CO₂ increases as the atmospheric concentration rises, but will this result in more tree growth or faster cycling of C through the forest? The question is critical because forests are prominent in the global C cycle and could provide an important negative feedback that slows the pace of climate change. Building on decades of research with seedlings and young trees, the current generation of experiments in forest stands confirms that net primary productivity, the total annual production of organic matter, should be higher in future forests. In the deciduous forest in the Oak Ridge free-air CO₂ enrichment experiment, productivity is enhanced by elevated CO₂ but the additional C is not recovered in tree biomass. Instead, it is allocated to fine roots that turn over rapidly and do not contribute to a removal of CO₂ from the atmosphere. Our challenge is to predict if other forests will allocate C similarly and how forest responses to elevated CO₂ will be modified by other resources and climatic change factors.

3.11.2. Ecosystem responses to increased atmospheric CO₂ concentration: Interactions with spatial and temporal variation in nitrogen and water

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In a *Pinus taeda* (L.) dominated ecosystem at the Duke Forest, spatial variation in leaf area index (*L*) corresponds to small topographic relief at the site (~20 m). In plots positioned at the higher portion of the landscape where site nitrogen (N) availability

is low, *L* of the planted pine had expanded slowly, reaching a lower maximum than plots positioned marginally lower at the site, and permitting higher proportion of canopy *L* from naturally regenerating broadleaf species. Nevertheless, absolute *L* of broadleaf species also increases with site N, but this increase is small due to heightened competition with the pine. In this setting, increasing atmospheric CO₂ (using Free Air CO₂ Enrichment, FACE) where N availability is low has no effect on *L* and growth; where N availability is high CO₂ enrichment enhances *L* and growth of the pine only. Inter-annual variation in water availability did not affect the above responses in four years of progressive water limitation, although drought reduces the absolute differences in leaf area among plots and treatments. However, the recovery from drought differs among species and depends on nitrogen availability and CO₂ treatment.

3.11.3. Plant-Microbe interactions in soil regulate ecosystem response to atmospheric CO₂

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A major uncertainty in predicting ecosystem response to a changing environment is the extent to which increases in plant and ecosystem productivity will be sustained as CO₂ continues to accumulate in the Earth's atmosphere. Here we present results from a major FACE experiment in Wisconsin that demonstrate how microbe interactions in soil mediate key feedback mechanisms controlling ecosystem response to elevated CO₂. Results demonstrate that increases in plant growth under elevated CO₂, and its diminishment by elevated O₃, have altered net primary productivity, fine root production and biochemistry, substrate availability for microbial metabolism, the composition of soil fungal communities and the formation of soil organic matter. We demonstrate how altered plant growth and tissue biochemistry under elevated CO₂ cascade through soil microbial communities to influence the ecosystem-level cycling of C and N in ecologically and economically important forests in the Upper Great Lakes region, USA.

3.11.4. Nutrients, rising CO₂ and carbon sequestration by the world's forests: a reality check

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Will carbon uptake by the world's forests partly counteract human CO₂ emissions over the next century, and thereby help mitigate climate change? Some suggest that it might, though often such predictions overestimate the biogeochemical flexibility of forest ecosystems. For example, increased nitrogen (N) fixation in response to rising atmospheric CO₂ has been invoked as a means to match potential carbon with N requirements of growing vegetation, yet in long-term field experiments, without additions of non-N fertilizers, CO₂ has no effect on N fixation. Similarly, in a woodland ecosystem, elevated CO₂ enhanced immobilization of N in plant biomass and soil organic matter, causing N availability to plants to decline. Based on specific examples from field experiments in woodland ecosystems, metaanalyses of published data, and biogeochemical theory, I argue that the expectation that forests will sequester large quantities of carbon is probably exaggerated.

3.11.5. Does exposure to elevated CO₂ delay the onset of senescence: Evidence from leaf, canopy and remote measurements in the POPFACE/EUROFACE Experiment.

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It has been suggested that the extended seasonality and delayed senescence observed in temperate forests, since the 1970s, is a consequence of increased ambient temperatures. Here, we present evidence to suggest a direct effect of elevated atmospheric CO₂ in mediating this effect in *Populus*. Using open-top chambers, we have shown that senescence is delayed in elevated CO₂ and have identified quantitative trait loci (QTL, areas of the *Populus* genome), responsible for this effect. In an ecosystem (FACE) experiment, we report evidence from leaf, canopy and remote measurements in *P. x euramericana* and *P. nigra* that senescence is also delayed in elevated CO₂. These include assessments of canopy and leaf reflectance spectroscopy,

(NDVI), Leaf area index (LAI) and leaf chlorophyll content suggesting that canopy longevity may be increased following exposure to elevated CO₂. The consequences of this for productivity and adaptation to future CO₂ will be considered, as will results from microarray analysis, where gene expression profiling has been completed as senescence progressed in both ambient and elevated CO₂.

3.11.6. CO₂ effects on juvenile tree stands, studied in 'mini-ecosystems'

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There is a strong need to conduct CO₂ enrichment experiments on plants at the community level under soil and microclimatic conditions as close to nature as possible. Therefore, several long-term experiments were conducted with juvenile *Fagus sylvatica* and *Quercus robur* stands in field enclosures ('mini-ecosystems') connected to soil containers of which the inner climate was regulated according to continuously working sensors outside. These systems not only allowed to measure total net CO₂/H₂O gas exchange of small stands together with rooted soil but also leaf and stem gas exchange and soil respiration. Selected results from several studies are compared in order to connect net photosynthesis and transpiration rates of leaves at elevated CO₂ with the total 'mini-ecosystem' gas exchange, with the aim to contribute to the answer on the question: Can woody plant systems serve as sinks for the additional tropospheric CO₂ and is there any interaction with their water use?

3.11.7. Effect of rising CO₂ on rainforest trees: Trends in ¹³C and water use efficiency of tropical *Cedrela* and *Swietenia* from Brazil

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Tropical forests are important sources (due to forest loss) and as sinks (through absorption by intact or re-growing forests) in the global carbon cycle. Without FACE experiments in any tropical forest, the effect of rising [CO₂] on an entire forest can only be studied through historical trends, such as those preserved in tree rings. While most rainforest trees do not produce annual rings, some do, and we present the first detailed analysis of long-term trends in C isotopes from two species from Brazil. Cellulose ¹³C from 37 *Cedrela odorata* (11 to 151 yr old) and 16 *Swietenia macrophylla* trees (48 - 126 yr) decreased substantially, with the strongest changes during the past 50 years. A comparison of cellulose from different decades and from trees of different cambial age showed that the trend observed is largely related to the decade the wood was produced in, and not to tree age. Internal CO₂ increased from about 220 to 260 ppm and intrinsic water use efficiency increased by 34% in *Cedrela* and by 52% in *Swietenia*. This may have implications for the water cycle and can also help to explain a trend towards increased tree growth and turnover observed in some tropical forests.

3.12.1. Phytoremediation technology for the management of trace element contaminated environment

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Phytoremediation technology for the management of trace element contaminated environment

Anthropogenic and natural flux of toxic trace elements are increasing in the environment deteriorating the quality of living. Phytoremediation technology includes a range of plant-operated processes that lead to the amelioration, removal, and containment of the toxic trace elements and operate on the principles of biogeochemical cycling. Various categories of plants viz, ornamentals, tree crops, aquatic macrophytes, algae, cell cultures, weeds, industrial crops are being applied in this strategy. This presentation would focus on the relevance of natural accumulators, molecular and genetic manipulation of plants, soil amendments with cheaters, interactions of soil organisms with higher plants, rhizosphere exudates for enhanced accumulation or exclusion of trace elements. Phytoremediation processes are in general slow and therefore is the general disbelief for its field applications on large scale. However, the contemporary

knowledge disproved this disbelief and phytoremediation technology is being transferred successfully for field applications.

3.12.2. Heavy metal uptake strategies of plant species growing on calamine (Pb/Zn) soil

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Some plant species on heavy metal enriched soils are able to influence uptake and distribution of heavy metals. Four species from lead-zinc enriched mine spoil on the Hochobir (Carinthia/Austria) were investigated. Plant heavy metal (Pb, Zn) content was determined using DCP-OES, distribution in different leaf tissues by means of EDXA. Leaf anatomy was examined by means of LM and SEM. Sequential extraction of the soil yields information on the mobility of the heavy metal fractions and the concentrations were measured with ICP-OES. In all plants lead concentration was highest in the roots. Regarding zinc distribution different strategies were observed: while *Linaria alpina* is an excluder type - most of the Zn is located in the roots. *Arabidopsis halleri*, *Thlaspi minimum* and *Alyssum ovirensense* are accumulators. The results show that in all analysed accumulator plants zinc is translocated in the aboveground organs; there are great differences, however, in distribution within several aerial parts (shoot, different leaf types) and tissues (epidermis vs parenchyma).

3.12.3. Plant-fungal interactions in hyperaccumulating plants

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Recently, the presence of arbuscular mycorrhizal symbiosis (including abundant formation of arbuscules) was demonstrated in Ni and As hyperaccumulating plants, which are being used in phytoextraction. In addition, in the case of *Berkheya coddii*, originating from Ni enriched ultramafic soils in South Africa, increased metal uptake was observed, which was due to the presence of a AM fungus. The most effective in stimulation of plant growth were strains originating from Ni-rich soils. The positive effect of a fungus was correlated with the richness of the developed arbuscules. This strongly suggests the importance of the appropriate selection of the strains. Both, in hyperaccumulating and nonaccumulating plants, AM fungi can either decrease or increase metal uptake. Diverse strains originating from several sites were used to estimate their influence on heavy metal uptake by plants. Under natural conditions, the fungi are not expected to increase strongly heavy metal uptake of these plants, due to the presence of both types of fungi, inhibiting and stimulating metal uptake. New technologies have to be developed to optimize the phytoextraction technique.

3.12.4. Cadmium induced changes in antioxidant enzymes in tobacco and coffee cell suspension cultures

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The contamination of the environment by heavy metal pollution is a serious problem. Oxidative damage often occurs due to the generation of oxygen reactive species induced by heavy metals. A class of antioxidant enzymes has been reported for the scavenging of the oxygen reactive species. We have studied the antioxidative responses of in vitro suspension cultures of tobacco and coffee to cadmium. It was observed alterations in CAT and peroxidases activities in BY-2 tobacco cells and an significant increase in GR activity. The results suggest that in BY-2 cells the main defence system to Cd stress is variable during the heavy metal exposure time. The synthesis of GSH is evident during the onset of the stress with CAT and peroxidases taking over the antioxidative responses once the stress becomes severe. For coffee cells, Cd exposure also induced an enzymatic oxidative response. The main response appears to be via the induction of SOD and CAT activities for the removal of ROS, or by the induction of GR to ensure the availability of reduced glutathione for the synthesis of

Cd-binding proteins (PCs). Financial support by FAPESP and CNPq.

3.12.5. Nutrients and heavy metal transfer from soil to the host-hemiparasitic plant association *Cistus-Odontites luteus* growing on mine spoils

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Odontites luteus (former Scrophulareaceae) a hemiparasitic plant was found associated with *Cistus* species at mine sites in Italy (Cu mine) and Catalonia (Pb, Ba mine). Soil, root and shoot concentrations of selected mineral nutrients (Ca, Mg, K, Fe, S, Mn, Zn, and Cu) and of non-essential elements (Al, Cd, Pb, Ba, and Sr) were analysed in order to get a first picture on how the hemiparasitic nutrient acquisition strategy may influence the mobility of these elements. *Cistus* sp. restricted the root to shoot transport of Cu, Zn, Al and Pb. In *O. luteus* similar root and shoot concentrations of Cu and Zn suggest that in this hemiparasitic plant the transport was less hampered, probably due to a direct uptake of mobile metal species from either or both xylem and symplastic sites of the host. In contrast, mobility of Pb was restricted also in the hemiparasite. The host-hemiparasitic plant association may serve as a model system for future investigations on metal mobility as a function of metal speciation in plants. *Acknowledgement: Supported by DGICYT (BFU2004-02237CO2-01) and Pla Recerca Catalunya 2001 (SGR00200).*

3.12.6. Toward the determination of the genetic basis of heavy metal accumulation in six wild metallicolous and non-metallicolous *Salix caprea* populations

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Due to fast growth and biomass production and the ability to accumulate high amount of cadmium (Cd) and zinc (Zn), *Salix caprea* is well studied for the clean up of metal-contaminated soils by phytoextraction. However only limited information on the population structure of *S. caprea* is available. Thus, analyses of the genetic structure of six metallicolous and non-metallicolous *S. caprea* populations was initiated. Genotyping 11 microsatellite markers, we found no evidence for clonal propagation. Low, but significant differentiation was observed between some *S. caprea* populations. By correlating the amount of heavy metal accumulation with the genotypes we anticipate to determine a genetic basis of this trait. To associate genes responsible for heavy metal accumulation, expression of candidate genes was quantified by real-time PCR during the vegetation period in leaves of *S. caprea* cultivated on contaminated and uncontaminated soil. The relationship between seasonal changes of gene expression as well as associations between the capacity of heavy metal accumulation and the genotypes will be discussed.

3.13.1. The roles of plant terpenoids in the environment and in the plant

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The smaller terpenes, isoprene, monoterpenes, and sesquiterpenes, play important roles in helping plants cope with stress. These compounds are made by related enzymes that are coded for by a rapidly-evolving family of genes. This has allowed these compounds to fill a variety of roles within plants including tolerance of, or resistance to, heat stress, oxidative stress, herbivory, and microbial attack. This talk will focus on the smallest isoprenoid, isoprene, and its role in protecting against thermal and oxidative stress. Isoprene does not protect against very high or sustained temperature and isoprene emission is uncommon in hot desert plants. However, plants that suffer frequent moderately high temperatures, such as tree leaves exposed to full sunlight on a still day, tend to emit substantial quantities of isoprene. Many trees routinely emit 2% of the carbon they take in for photosynthesis as isoprene, but this can range to over 50% of carbon lost as isoprene. Leaves at the tops of trees emit four times more than leaves at the bottom of the same tree. The evolution of the capacity for isoprene emission, and the atmospheric consequences will be addressed.

3.13.2. The biology and molecular genetics of terpene metabolism in plants

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Terpenes are a structurally diverse class of compounds that play important biological roles ranging from antimicrobial defense compounds to hormones controlling development programs in plants. Interestingly, terpene biosynthesis has only been observed in plants, microbes and insects, but not in animal species. In plants, terpenes are synthesized by 2 complex biosynthetic pathways compartmentalized to the cytoplasm and the plastids. Recent progress in understanding the structure and function of the terpene biosynthetic genes contributing to the cytosolic pathway has advanced our appreciation for the biological significance of these compounds in plants, as well as suggested mechanisms for how these genes may have evolved and been adapted for specific functions. Understanding the biochemistry and molecular biology of terpene metabolism has also provided insights into how these pathways may be engineered for practical applications in agriculture and medicine.

3.13.3. Regulatory aspects of isoprene formation in grey poplar (*Populus x canescens*) leaves

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Trees produce a wide spectrum of volatile organic compounds (VOCs) such as isoprene. Recently much progress was made to elucidate the metabolic pathway leading to isoprene. Two possible committing steps in the plastidic pathway are the deoxyxululose 5-phosphate (DOXP) reductoisomerase (DXR) catalysing the first specific reaction of the methylerythritol phosphate (MEP)-pathway and isoprene synthase (ISPS), the final enzyme releasing isoprene from dimethylallyl diphosphate (DMADP). The presentation summarizes actual work on molecular, biochemical and physiological aspects of isoprene formation in order to understand its biological function. Seasonal and daily variations of gene expression rates of *dxr* and *ispS* in relation to enzyme activity, DMADP pools and isoprene emission rates from poplar will be shown demonstrating the high variability of isoprene formation. Furthermore immunological and immunohistochemical data on the tissue-specific localisation of ISPS will be presented.

3.13.4. Plant terpenoids in information webs: the ecology of 'crying' for help

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In response to herbivory plants produce volatiles that attract the enemies of the herbivores as a kind of 'bodyguards'. Terpenoids are a major class of herbivore-induced plant volatiles that have been recorded from a large number of plant species in response to herbivory by e.g. caterpillars, mites, beetles or bugs. The volatiles that a plant releases can be exploited by all organisms in the environment, including herbivores and neighbouring plants. Therefore, the emission of herbivore-induced plant volatiles may alter various interactions in the food web and in the information web. In this presentation a link will be made between chemical ecology, molecular biology and community ecology. More and more terpene synthase genes are being cloned and characterized and these genes allow exciting new options to investigate the role of terpenoids in multitrophic interactions. Novel tools that allow major progress include combining metabolomics and genomics with ecology. This will be illustrated with research on cucumber and Arabidopsis.

3.13.5. Modeling the emission of stored and "non-stored" monoterpenes from *Pinus pinea*

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We developed a dynamic model to describe time-dependent changes in VOC emissions from the foliage of Mediterranean conifer *Pinus pinea*. The model consists of a storage pool in resin ducts and two nonspecific monoterpene storage pools in leaf liquid and lipid phases. The monoterpene emission is further controlled by stomatal conductance, that is reflected in a dynamic stomatal

control term. The model was parameterized using laboratory monoterpene ^{13}C labeling- and delabeling data. The model is capable to map the dynamics given by the measured ^{13}C labeling data, and demonstrates that the emissions from the non-specific storage pools dominate the monoterpene emission in light. The model further demonstrates that the composition of emitted monoterpenes is dependent on monoterpene physico-chemical characteristics. We conclude that non-specific monoterpene storage pools should be included in monoterpene emission models in both the species possessing and lacking specific foliar monoterpene storage structures.

3.13.6. Isoprene and other terpenoids in the marine environment

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Isoprene and other terpenoids are produced in the oceans by photosynthetic algae. While differences exist between marine and terrestrial terpene biosynthesis, isoprene production appears to occur through similar mechanisms in both locales. Environmental and physiological parameters such as light, temperature, and chlorophyll content affect isoprene production from phytoplankton and seaweeds. Bacteria and grazers do not directly produce or consume isoprene, but virally-infected phytoplankton have reduced production rates. Possible functions of isoprene to phytoplankton and seaweeds will be discussed. Air-sea isoprene fluxes can affect tropospheric oxidant chemistry and aerosol formation. New satellite-based global marine flux estimates will be compared with the limited in situ measurements available. Recent field campaigns show that iron fertilization can significantly increase marine isoprene fluxes above background values.

3.13.7. Seasonal terpene emission variation investigated for monoterpene emissions from European beech (*Fagus sylvatica* L.)

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In our study we investigated the terpene emissions from European beech (*Fagus sylvatica* L.) in a deciduous forest. Applying the branch enclosure technique changes in the emission pattern and the variation of emission rates over the year were investigated for two following vegetation periods. Our results show that European beech emits significant amounts of monoterpenes, especially sabinene. Monoterpenes have strong impact on the processes in atmospheric chemistry and in inter- and intra-specific signal transmission of plants. The overall emission pattern of beech differed only slightly while emission rates changed up to two orders of magnitude as a function of temperature and light over the day. Highest emission rates were observed in summer and lowest in fall. However, no emissions were found in early spring although leaves were fully developed and temperature and light conditions were comparable to the conditions in fall. Moreover, a temperature independent decline of emissions in late summer characterizing a seasonality of terpene emissions was found resulting in changes of the standard emission rate on the order of one magnitude.

3.14.1. Quantifying the role of dispersal, ecological drift, and species sorting in determining the diversity of local communities

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Metacommunity theory shows that regional dynamics can have considerable consequences for the diversity and composition of local communities. However, whereas the theory explores the role of dispersal in *maintaining* diversity within communities, empirical ecologists have largely focused on the role of dispersal in *limiting* local diversity. I argue that there is no necessary relationship between the two; hence we actually do not have the empirical data needed to test and quantify how regional and local processes interact to determine local diversity. Here I present an empirical approach that can be used to explore these questions. It is based on a quantitative partitioning of the natural regeneration within intact communities onto different sources of recruits (e.g., local vs. dispersed). Recruits from these different sources are then followed over time to assess the relative importance of local processes (e.g., species sorting vs. ecological drift) in determining diversity of the adult vegetation. Finally, species traits can be used to determine

whether species sorting results from life-history trade-offs or habitat niche differences among species.

3.14.2. Invasibility, propagule availability and diversity of an alpine plant community under simulated climate change

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Which factors control alpine plant community invasibility and diversity under natural conditions, and how will climate warming affect colonization processes and diversity? We examine the relative role of propagule availability and community-level interactions for the invasibility and diversity of a high-diversity plant community in alpine Norway, under natural and elevated temperature conditions. Adding propagules from 27 regional species into heath vegetation increased community diversity significantly. High resident species richness and cover of the highly abundant *Dryas* reduced invasibility. Propagule limitation and competitive exclusion from established species controlled community invasibility and diversity more than the availability of safe sites and the experimental warming. The establishment success of the 'invaders' increased under elevated temperature, but intensified competition from established species on emerging seedlings under experimental warming suggest that climate change effects on biotic interactions may affect long-term colonization processes and diversity of alpine plant communities.

3.14.3. Competition-colonization trade-offs and seed limitation in tropical forests

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Competition-colonization trade-offs between species' ability to arrive at sites and to win sites at which they arrive may contribute to diversity maintenance in stabilizing (niche-y) or merely equalizing (neutral) ways, depending on the specifics of the trade-off and the degree of competitive asymmetry in the community. Alpha and beta diversity patterns are also affected by species dispersal distances in general, even absent any interspecific differences or trade-offs. We use 18 years of seed rain data within a diverse tropical forest to quantify seed production, seed dispersal distance, and seed limitation for 80 tree species, and 11 years of seedling dynamics data to evaluate species' competitive abilities and competitive structure. Seed limitation is very strong for most species, and varies widely among species due to large differences in seed production and lesser differences in dispersal distances and clumping of seed rain. Seed size mediates a trade-off between seed production and competitive ability among species. We investigate the potential of the observed competition-colonization trade-off and seed limitation to contribute to diversity.

3.14.4. Competition, colonization, or herbivory: which explains patterns of dominance in herbaceous communities

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We seek to offer a unified and mechanistic explanation for the dominance of goldenrods (*Solidago*) in herbaceous communities across vast regions of the US. Goldenrods coexist with numerous other long-lived herbaceous species, yet these other species rarely become dominant. We are conducting one of the first ever field tests of the predictions of mechanistic resource competition theory while simultaneously evaluating alternative explanations for dominance, specifically, colonization ability and herbivore tolerance. We ask the simple question: Are species abundant because they are superior competitors, superior colonizers, or resistant to herbivores? Our goal is to determine whether species' traits (e.g., colonization ability) are capable of explaining the mechanism underlying patterns of relative abundance. Alternatively, traits may not provide a viable explanation if species share many of the same plant traits. Our findings to date suggest that goldenrods dominate herbaceous communities because relative to the species they coexist with, they are simultaneously the best resource competitors, the best colonizers and are unpalatable to browsers.

3.14.5. Roles of chance and adaptation in the assembly of fern communities

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We evaluated correlations among fern distributions, the underlying abiotic environment, and the spatial proximity of sampling points at meso- and fine-scales (135-3515 m and 4-134 m, respectively) within an old-growth, deciduous forest. Moisture availability contributed significantly to fern distribution at both spatial scales, and species kept the same positions along the moisture gradient across scales. Dispersal had no significant role in determining meso-scale distributions of fern species. At the fine-scale, however, the environment was strongly spatially structured, and species distributions along moisture gradients might just as well have arisen in neutral dispersal processes as through any niche requirements. Such spatially structured local environments may foster the persistence of dispersal-limited plants. While neutral or random models of species coexistence cannot explain the patterns we have observed, any deterministic models that rely on strict niche requirements without accounting for local dispersal and the inherent spatial structure of the environment neglect the important interaction of these factors.

3.14.6. Plant community assembly and the unimodal productivity-diversity relationship

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The unimodal (hump-backed) productivity-diversity relationship was first described by J. P. Grime for British herbaceous vegetation. The original explanation claimed that smaller diversity at low productivity is due to abiotic stress, while at high productivity, competition (niche limitation) is decreasing diversity. Alternatively, evolutionary-historical mechanisms (the species pool size may be small for low or high productivity habitats) and dispersal limitation (diaspores of proper species do not arrive the community) may limit local diversity. Seed addition experiments in grasslands have increased diversity mainly in unproductive habitats, indicating that both dispersal and niche assembly may limit local diversity, but in different parts of productivity gradient. We studied published evidences on productivity-diversity relationships and found that unimodal relationship was restricted to herbaceous vegetation and cold climate. Thus, besides the productivity gradient, life-form specific ecological mechanisms, and regional evolutionary history may determine the balance between dispersal and niche limitation in plant communities.

3.14.7. Quantitative partitioning of regional and local processes shaping diversity productivity-diversity relationships

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Much recent research in explaining plant community diversity has focused on quantifying the relative impacts of regional and local processes. These analyses have been used successfully within communities, but the power to generalize must come from investigations of patterns of diversity across communities. We employed a novel analysis to integrate multiple regional and local processes to explain patterns in species richness across two plant communities that differ in productivity. While abiotic stress and competition appeared to limit richness within both communities, only differences in species pools resulting from long-distance dispersal and climate fluctuations explained the higher richness in the less productive community. By identifying both the factors that impact diversity within communities and those that vary systematically across communities, our integrated approach provides a better understanding of regional diversity patterns and a stronger basis for predicting community responses to environmental change.

3.15.1. Ozone in Europe: Distribution and effects on plants in the Carpathian mountain and Southern Alpine regions

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Ozone has become the major phytotoxic air pollutant in Europe. Researchers from the USA and Europe have determined the distribution and effects of ozone in forested and natural areas in the Czech Republic, Poland, Romania, Slovakia and Ukraine, in the Carpathian Mountain region and in Italy, Spain and Switzerland in the Southern Alpine region. There is evidence that ambient ozone concentrations as they are monitored in Central and Eastern Europe can cause a variety of effects to vegetation such as visible foliar injury, growth and yield reductions, and altered sensitivity to biotic and abiotic stresses. Ozone may cause shifts in species composition and loss of biodiversity in forest and grassland ecosystems. It is often difficult to assess the sensitivity of natural ecosystems to ozone, especially when considering the diversity of sites as they are found across the Carpathian and Southern Alpine regions. The current international efforts for the establishment of a biologically more meaningful ozone standard demonstrate that more data for dose-response relationships are needed in order to enable ozone risk assessment for natural plant ecosystems.

3.15.2. Ozone in Europe: distribution and temporal trends in the Carpathian region

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Ambient ozone (O₃) concentrations are highly variable in the Carpathian Region. Some areas of the Carpathian Mountains in Romania and Poland, as well as the Sumava and Brdy Mountains in the Czech Republic are characterized by low concentrations of the pollutant (seasonal mean concentrations ~30 ppb). Other parts of the Carpathians, especially western part of the range (Slovakia, Czech Republic and Poland), portions of the Eastern Carpathians (Ukraine) and Southern Carpathians (Romania), and the Jizerske Mountains in the Czech Republic have high O₃ levels (peak values >100 ppb and seasonal means ~50 ppb). Large portions of the central European mountain forests experience O₃ exposures above those recommended for protection of forest and natural vegetation (AOT40 values of 10,000 ppb x h and 3,000 ppb x h, respectively). The most endangered forests are in the Slovak parts of the Carpathian Mountains and in the Jizerske Hory of the Czech Republic. There is need for continuation of ambient O₃ monitoring efforts with a combination of active monitors and passive samplers for understanding risk from ambient O₃ in forests and other ecosystems of the Carpathian Region.

3.15.3. Plants and plant ecosystems at risk in the Carpathian mountain region

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The Carpathian Mountains represent one of the most significant natural regions in Europe. They are characterized by high richness of plant species (30% of the total European flora) and unique natural and seminatural ecosystems. The Carpathians are recognized as a biosphere core areas of an European significance, providing important ecological corridors for the pan-European migration routes. More than 50% of the Carpathian territory is forested, arable lands, pastures and meadows occupy 29%, alpine meadows and rocks 15%. The Carpathians have been under direct (management) and indirect (pollution) human impact for a long time. SO₂, NO_x and dust emissions have been affected the Carpathian ecosystems since the last 50-60 years, recently they are affected by increasing concentrations of ozone (O₃). Consequences of human activity for the biodiversity and health of the Carpathian ecosystems as well as preservation policy and protection of the Carpathian landscape will be presented.

3.15.4. Plant response to ozone in the Carpathian mountain region

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Ozone has become an all-pervasive phytotoxic air pollutant in the Carpathian Mountain regions of the Czech Republic, Poland, Romania, Slovakia and Ukraine in Central and Eastern Europe. In warm months, increases in combustion, mainly from more cars and trucks, accelerates the photochemical oxidant cycle to produce ozone. Long-range transport from Western Europe also contributes to higher ozone levels in the region. In areas where ozone is known to exceed natural background levels, sensitive native plants may be injured by ozone. We found twenty-nine species of plants in natural areas that exhibited ozone-like injury. By exposing plants to ozone under controlled conditions, we were able to verify 8 forbs (*Alchemilla* spp., *Astrantia major*, *Centaurea nigra*, *Centaurea scabiosa*, *Impatiens parviflora*, *Lapsana communis*, *Rumex acetosa*, and *Scenecio subalpinus*), 3 shrubs (*Coryllus avellana*, *Cornus sanguinea*, and *Sambucus racemosa*), 3 trees (*Alnus incana*, *Pinus cembra*, and *Sorbus aucuparia*) and two vines (*Humulus lupulus* and *Parthenocissus quinquefolia*) as detector bioindicator plants that can be used to indicate relative air quality for ambient ozone

3.15.5. Ozone in Europe: Distribution, temporal trends and physiological effects on plants in the Southern Alpine region

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Numerous studies have shown that tropospheric ozone can have negative effects on forest plant species in many parts of Europe including the southern Alpine region, where ozone exposure frequently exceed the critical levels set to protect forest vegetation. These high ozone exposures occur throughout southern Switzerland and northern Italy and are not limited to urban and industrial centers, but often occur in rural areas many kilometers from the source of the precursor pollutants. A variety of studies have been conducted in open-top chamber facilities, in controlled laboratory conditions, and under open field conditions and both morphological and physiological effects have been observed on a variety of ozone-sensitive plants species native to this region as well as much of Europe. As ozone exposures continue to annually exceed the set critical levels, ongoing research focuses on addressing the question as to how ozone affects forests on a regional scale. This paper aims to provide an overview of more than a decade of ozone research as well as the ongoing research conducted in the southern Alpine region.

3.15.6. Ozone sensitivity of temperate grasslands

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Grasslands cover a major part of European landscapes, and they are important for both agricultural utilization and the conservation of biodiversity. With their long growing season, their exposure to air pollutants, mainly tropospheric ozone, is high. The resulting long-term effects may include species-specific reductions in productivity or reproduction, which may lead to a change in species dominance or even a loss of genetic or species diversity. Recent studies using single plants, mesocosms, or intact communities confirmed that ozone affects individual species, and their interactions. This review indicates that the magnitude of ozone effects observed in experiments and obtained by modelling varies according to the degree of system complexity and the duration of the exposure. Effects tend to decrease over time, and complex communities tend to react less than individual plants or simple mixtures. However, further work with established grassland communities will be necessary to fully assess the risks of exposure to ozone.

3.15.7. Validation of the 'unifying theory' on O₃ sensitivity in adult forest trees of Central Europe (*Fagus sylvatica* versus *Picea abies*)

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Reich (1987, *Plant Physiology* 3: 63-91) claimed plant responses to chronic O₃ stress to be explained through a "unifying theory" based on leaf diffusive conductance. Convergence was suggested per equivalent total O₃ uptake or uptake per leaf life span, although validation for adult forest trees has been lacking so far. Here, experimental validation is exemplified for beech versus spruce under ambient air or enhanced O₃ impact through free-air fumigation. Given actual growing seasons and leaf longevities as well as age and O₃ effects, spruce required, per leaf surface area, 5 years for the O₃ uptake of beech during one growing season. The "unifying theory" was supported as both species displayed similar proportional decline in photosynthesis in relation to cumulative O₃ exposure and uptake per "leaf lifespan day". Non-linearity relative to O₃ flux rather than AOT40 extends the scope towards O₃ detoxification and repair. Overall, spruce was not inherently less O₃-sensitive than beech.

3.16.1. Save it, use it, share it - biodiversity as boundary object

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In this paper I analyse the concept of biodiversity from the perspective of science studies. Its invention and establishment are interpreted as particular interests to professionalize environmental concern. As the concept and politics of biodiversity transcend traditional boundaries between science and society and between facts and values, its invention is interpreted as boundary work, as an effort to redefine the legitimate boundaries of science in ways that include evaluative statements and political practices. After analysing the interests of the involved scientists the focus of the paper shifts towards the political context. In reconstructing the different interests that were involved and finally addressed in the Convention on Biodiversity I show that biodiversity can meaningfully be regarded as a boundary object, i.e. an object that is able to co-ordinate different groups without a consensus about their aims and interests.

3.16.2. Biodiversity governance between sustainable use and biopiracy

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The term Biodiversity is constituted in two distinct ways. At the one hand its meaning is directed towards a comprehensive understanding of one of the most important global environmental problems. At the other hand its recent career is connected with the economic value of genetic resources, being the raw material of new biotechnology companies. Over the last years an international regulation system was build up to create the political-institutional framework for the sustainable use of Biodiversity. But this governance system is neither an efficient framework for Biodiversity conservation nor an effective political framework to regulate the functional prerequisites of global markets of genetic resources. At the contrary, it is a highly contradictory and contested network established by a number of international institutions and organisations. In the contribution, based on a research project on the global management of genetic resources, this governance structure is analysed towards the question whether it allows a fair and equitable sharing of benefits arising from its use - or whether it favour Biopiracy, as some activist group argue.

3.16.3. A new international regime - practical issues for botanists

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A new International Regime (IR) on access to genetic resources and benefit-sharing is now being negotiated under the Convention on Biological Diversity. Proposals include mandatory disclosure of the country of origin in applications for intellectual property rights and a system of certificates of origin/legal provenance/source for genetic material. What will the practical implications of the IR be for researchers and institutions, and how can we help shape the outcome?

If any new system is to sustain rather than hinder biodiversity research and conservation, the research community to be proactive and involved in its development. Biologists need to communicate with peers, partners and government negotiators, and also to demonstrate that our work is worthy of support not suspicion. We need to re-examine how we: collect plants with appropriate consent; collaborate; ensure permit terms stay linked to material we curate, use and exchange; share benefits; disseminate information; and raise awareness. We will provide an update on the start of IR negotiations (February 2005) and give examples of practical measures at the Royal Botanic Gardens, Kew.

3.16.4. Images of nature: Connecting scientific knowledge and the valuation of biodiversity - a case study from southern Chile

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Images of nature are expressions of people's perception of nature in which facts and values, experiences and cognitive knowledge are still closely connected. For this reason they can form an interface for interdisciplinary and participatory work on the evaluation of biodiversity. Since July 2003, these issues are investigated in the course of a German-Chilean research project in the very south of Chile (BIOKONCHIL). The region is one of the few not fragmented temperate forests of the world and is characterized by high biological diversity and naturalness. On the other hand time economic pressures, such as salmon farming or intensive tourism are recently threatening biodiversity in the region. The paper will present the methodological approach and first results of the project. The project uses ecological theory and the analysis of images of nature to connect scientific knowledge and people's perception of biodiversity. The aim is to provide insights into the valuation of biodiversity from different perspectives (ecological, ethical, economic). Ultimately this serves to provide supportive information for the decision process of the region's development.

3.16.5. Monitoring biodiversity, the quadrature of the circle for biologists. a workshop report from Austria

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An indicator set for monitoring biodiversity has to meet very diverse demands: - comply to international commitments, not only the CBD but also the different tasks burdened by the EU on its member states and - match already existing international examples, particularly EU guidelines (which are in statu nascendi), - be specially designed for Austrian conditions and necessities; - directly connected to pressures threaten biodiversity or measures that have an influence on biodiversity; - based on sound science and statistically valid data, - but also fostering a system oriented, transdisciplinary approach with results that are comprehensible and convincing to everybody, - altogether an approach that is conservative but also selfreflexive and innovative as well, - and will last but not least not only look fine on paper but will be really useful for the promotion of all forms of BD on all levels, - and is of course inexpensive, because the money should be used for action rather than mere monitoring. A bold team of experts has tackled the task to design such a miraculous indicator set. As their project will be already in an advanced stage in July, let us see how they succeeded.

3.16.6. World bank and forest conservation in India

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The conservation of the forest by the 'State Forest Department' of the Government of India is a bound duty. The same become either strengthened or weakened due to the involvement of foreign

monetary aids. During the last couple of years the involvement of some World Bank aided projects in a few states of India reveals an unprecedented outcome in this matter. The present paper represents a case study undertaken in the western state of India with the aim to evaluate the impact of such punctual monetary funding towards local forest conservation. This study does indicate that the concept of conservation is being well appreciated and supported by the regional population, however there remains a big feeling of insecurity among the local people leading to the unsaid conflict between the forest and its dwellers.

3.17.1. Biologically active plant compounds in food

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Phytonutrients and phytochemicals are commonly referred by nutritionists to biologically active compounds naturally occurring in plants that have physiological effects in human and might prevent or delay chronic degenerative diseases and enhance human health. Numerous phytochemicals have been identified as components of edible plant food with special focus on polyphenols, flavonoids, anthocyanins, carotenoids, isoflavones and lignans. Most that have been studied have in common an ability to act as antioxidants and free radical scavengers to prevent cell damage and are associated with reduced risk of cancer, cardiovascular disease and help prevent and/or treat other medical ailments. They are found in a number of foods, e.g. soy, legumes, fruits, berries, vegetables, seeds, tea, grapes and wine, representing an important factor to consider in the formulation of functional foods and in the choice of a healthy diet. Future food trends will be aimed at modification and fortification of food to enhance naturally phytochemical concentrations for incorporation in the diet for the benefit of the consumer.

3.17.2. Functional food - relevance and trends in nutrition

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A change in paradigm has occurred: food is now seen also as a tool for better health rather than only to cover energy needs. Definitions are given, differences are pointed out. The legal situation in the EU is stressed. Functional Food Science offers new insights to the interrelationship between food and health. The proof lies in the discovery of beneficial effects of food(components). The scientific background of the claims and trends in Europe are outlined. The tendency to use functional ingredients as food supplements is stressed. Health claims play an important role on the market. Examples are given and the dose - response relationship and potential overdosing is discussed. In any instance food quality - which also comprises the health or beneficial effect - is a dynamic concept, subjected to sociological changes and perception of new scientific knowledge, which is defined according to a personal ranking order of values

3.17.3. Purple wheat - a raw material with high content of phytonutrients

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Current interest in the health benefits provided by grain consumption has led to an increased focus on the phytochemical content of different grain varieties. Among these, special attention is paid to purple or blue varieties which contain additionally anthocyanins. The objectives of this study were to determine the phytochemical content and reducing power of purple wheat (various milling fractions) and purple wheat products (bread, wafers and pasta) from two diverse varieties in comparison to one normal quality wheat variety. The phytochemical content was characterised by total phenolic content, total anthocyanin content and yellow pigment content. Results demonstrated that purple wheat contained more secondary plant metabolites which resulted in a higher reducing power compared to conventional wheat. Thus, purple wheat is an interesting potential source for innovative cereal products within the health food market.

3.17.4. Anthocyanes from edible berries - pigments with physiological effects

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Anthocyanins are water soluble substances from plants, mainly from fruits, that are responsible for the cyanic colors of the fruits. Due to the phenolic structure the anthocyanins are strong antioxidants having the potential to reduce the risk of degenerating diseases where oxidative reactions are involved. On basis of these preliminary results a double blind placebo controlled study was conducted to show the effect of the elderberry juice on the cholesterol level. The results showed a small effect on the serum cholesterol. Other blood parameters were not affected. The effect on the cholesterol concentration was not significant. The antioxidant effect of anthocyanins was not confirmed in serum after oral uptake of the elderberry juice. The pharmacokinetics of the anthocyanins from elderberry were analysed in serum. It could be shown that the maximum concentration of the anthocyanins in the serum after ingestion of the powder was reached after 30 min. After 7 h the anthocyanins were cleared from the serum. Using a lipid rich meal prior to the uptake of the elderberry juice the level in the serum was much slower.

3.17.5. Antioxidant properties of ethanolic extracts from holy basil and galangal and its application to inhibit lipid oxidation in cooked minced meat

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Ethanolic extracts from Holy basil and Galangal were prepared and evaluated for their antioxidant properties and their effects on lipid oxidation in cooked minced meat during storage at 5 °C. The ethanolic extracts of Holy basil and Galangal had good thermal (80°C, 1 h) and pH stability. The DPPH radical scavenging activities of both extracts were high and showed antioxidant activity, superoxide anion scavenging activity and reducing power in a concentration-dependent manner. Therefore, this extracts possibly worked as primary antioxidants that reacted with free radical and oxygen radical. Holy basil and Galangal extracts function as Fe²⁺ chelator and also inhibited lipoxygenase activity. Furthermore, ethanolic extracts from Holy basil and Galangal inhibited the lipid oxidation in cooked minced meat during storage at 5 °C by reducing both peroxide value (POV) and 2-thiobarbituric acid-reactive substances (TBARS). Thus, this extracts may be a possible natural antioxidant source for meat and meat products.

3.17.6. Increases of human plasma antioxidant capacity after consumption of controlled diets high in the content of anthocyanins, flavonoids and other phenolics

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Objectives: The objective of this study was to investigate whether a diet of fruits and vegetables would affect the antioxidant capacity of human plasma. Design: Twenty healthy persons consumed two sets of controlled diets. Diet A contained eleven servings of fruits and vegetables each day for seventeen days. Diet B was the same as Diet A, except Diet B provided three servings of kale and blueberries for days six through eleven. There was a free-living period of seven weeks between the two experiments using either Diet A or Diet B. Fasting plasma antioxidant capacity, measured as ORAC, alpha-tocopherol and ascorbic acid were determined with HPLC-ECD on days 1, 6, 12 and 18. Hippuric acid in the urine was measured by a photometric method. Results: Plasma ORAC, alpha-tocopherol, ascorbic acid and urine hippuric acid of these persons were significantly increased by diet A, but more by diet B. Conclusion: Increased consumption of fruits and vegetables with high contents of phenolics, flavonoids, anthocyanins and vitamins can increase the plasma antioxidant capacity and the excretions of hippuric acid in the urine of humans

3.17.7. Evaluation of in vitro estrogenic activity of some Fabaceae species.

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The estrogenic activity of plants is related to the presence of compounds referred to as phytoestrogens, mainly isoflavonoids,

lignans and cumestans; however other substances have been found to bind to the human estrogen receptors. Based on recent literature, foods rich in such substances, beside having effects on the reproductive system, seem to play an important role in chronic degenerative disease prevention. Thus the evaluation of the estrogenic activity of foods belonging to the traditional nutritional habits of populations with a low incidence of cardiovascular and hormone-dependent cancer, may contribute to new strategies for better population health. In this work we have analysed the *in vitro* estrogenic activity of crude extracts of many *Fabaceae* species largely used as food in Mediterranean and Oriental tradition, using an assay based on engineered human cells expressing the estrogen receptors and a reporter gene. Results indicate that many *Fabaceae* show estrogenic activity, mainly present in the more polar extracts; however consistent differences were observed in related species and among varieties of the same species.

4.1.1. D-type cyclins: Dual roles in G1/S control and development in *Arabidopsis*

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In plants, control of the cell cycle is of key importance in the activity of meristems, developmental processes and the overall control of growth. *Arabidopsis* has ten D-type cyclin genes (CYCD), and the use of synchronised cell suspension cultures shows that these are expressed with distinct timing during cell cycle re-entry and during the cell cycle. We have focussed on CYCD3 as a key regulator both of the G1/S transition, and of differentiation and endoreduplication. CYCD3 is an unstable protein, rapidly turned over in a proteasome-dependant manner, and overexpression in suspension cells shows that it drives cell through the G1/S boundary but this causes an extension of G2 phase before the point at which B-type cyclin expression is activated. CYCD3;1 is highly expressed in developing primordia, and we propose that the primary role of CYCD3;1 is to maintain cells in a mitotic cycling state and to block endoreduplication until sufficient cells have been produced in a developing organ.

4.1.2. Complexities of the E2F-regulated gene expression networks during the cell cycle and differentiation

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The balance between cell proliferation and differentiation is crucial in multicellular organisms but particularly relevant in plants where organogenesis is a postembryonic process. Maintenance of this balance requires a complex and finely tuned transcriptional regulation, both temporally and spatially. The E2F/DP family of transcription factors was originally considered to be involved exclusively in regulating G1/S genes. Recent evidence has revealed that, surprisingly, this is not the case, since they actually regulate genes that belong to many different functional categories. We are interested in identifying set of genes that are preferentially regulated by different E2F in a cell type- and developmental stage-specific manner. Among them, those involved in controlling the initiation of DNA replication and macromolecular biosynthesis play important roles in the maintenance of proliferative potential, the switch to the endocycle program, the acquisition of a differentiated state and, ultimately, the correct morphogenetic patterns.

4.1.3. Recombination-replication-repair connection of geminiviruses

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Geminiviruses are single-stranded (ss) DNA-containing plant pathogens. One major driving force of their evolution is recombination. Using an optimized two-dimensional gel electrophoresis in combination with hybridization and electron microscopy, we have discovered that the recombinational flexibility is reasoned by a recombination-dependent replication mode (RDR). Compared to complementary strand synthesis (CSR) and rolling circle replication (RCR), which both accompany RDR, the latter mechanism allows the virus to repair every broken or unfinished DNA intermediate as far as homologous templates are available. Surveying different combinations of geminiviral and satellite DNAs, two strategies can be discriminated between Old World and New World begomoviruses. The first is rather

promiscuous and transreplicates other DNAs without a cognate DNA-binding sequence for the viral replication-associated protein (Rep), whereas the latter needs the interaction of Rep and Rep-binding sequences.

4.1.4. Regulation of the transition from G2 to mitosis in higher plants

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The classic eukaryote cell cycle comprises mitosis (M) and DNA replication which are separated in time by G1 and G2. In effect, it is the conduit through which proliferative cells travel from one mitosis to the next. We study the transition from G2 to mitosis and in this paper I will describe what we know about this transition at the molecular level. In plants, the key regulators of this transition are A-type cyclin dependent kinases (CDKs), A-type cyclins, B-type CDKs /cyclin Bs. CDKAs are expressed from S-phase to G2 whilst, only in G2 are plant-specific B-type CDKs expressed. A-type CDKs but not B-types can complement budding and fission yeast mutants *cdc28* and *cdc2*, respectively. B-type CDKs also differ in that a conserved sequence PSTAIRE (A-types) in the helix 1 reads as PPTLARE / PPTTLRE in B-types. At G2/M, in fission yeast the Cdc2-cyclin complex is phosphoregulated by WEE1 kinase competing with CDC25 phosphatase at an ATP binding domain of Cdc2. In higher plants only recently, have putative phosphoregulators of CDKs emerged. We have cloned a full length *Arath*;WEE1 and a small *Arath*;CDC25 and are now testing the efficacy of these genes as cell cycle regulators.

4.1.5. DNA endo-reduplication - bypassing checkpoints and control points

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DNA endo-reduplication, the repeated replication of DNA in the absence of mitosis, is common in plants, so common in fact that it should be regarded as a normal cell cycle variant. Endo-reduplication can only happen if the checkpoints that link M to the subsequent S-phase are suppressed and if the mitotic control point is completely by-passed. In tobacco BY2 cells, mitotic cell division is inhibited by withdrawing auxin. However, after a lag of about three days, the cells start to elongate and then initiate DNA endo-reduplication. This implies that the molecular events required for DNA replication are induced in this auxin-minus growth pattern. Analysis of mRNA populations by RT-PCR indicates that the expression of the gene encoding CDC6, a protein required for initiation of DNA replication, is induced under these conditions. This may be compared with the 'normal' cell cycle that occurs in the presence of auxin, in which the expression of this gene is almost entirely confined to late G1 and to S-phase. Exactly what the role of auxin is in that pattern of expression is not clear, because the endo-reduplication data show that *CDC6* expression may be auxin-independent.

4.1.6. DNA endoreduplication during caryopsis development in sorghum (*Sorghum bicolor*).

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We report here temporal and spatial occurrence of endoreduplication (ERD, a form of polyploidization in somatic cells of many eukaryotes) in developing caryopsis of sorghum. After a short phase of intense mitotic activity and cellularization, endosperm cells showed ERD as early as 5 days after pollination (DAP), that peaked at 11 DAP in nuclei in the central region of endosperm. The highest measured C-values in those cells were 96 C (1 C represents the nuclear DNA content of a non-replicated haploid genome). A positive correlation was seen between the nuclear volume and the nuclear C-values. Positive correlation was also observed among the nuclear DNA content, volume of endosperm cells and the starch content. In the later stages of

development, approximately 70% of variance of endosperm cell volume could be explained by ERD. Nuclei with non-ERD DNA were observed exclusively in the peripheral layers of the endosperm. ERD was also prominent in the maternal pericarp nuclei, with DNA content up to 16 C.

4.2.1. Control of chloroplast gene expression in *Chlamydomonas*

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Chloroplast biogenesis requires the concerted action of two genomes, in the plastid and in the nucleus. Genetic analysis in the green alga *Chlamydomonas reinhardtii* has revealed a large number of factors that are encoded in the nucleus and are required for the expression of chloroplast genes. These factors are involved in post-transcriptional steps of gene expression such as RNA processing, splicing, translation or assembly of protein complexes. They show a surprising specificity for single chloroplast genes or gene clusters. A striking example comes from the chloroplast *psaA* gene of *Chlamydomonas* which contains two split group II introns. The three exons are widely scattered in the plastid genome and are transcribed as separate precursors that are then spliced *in trans*. The maturation of *psaA* mRNA depends on the products of at least fourteen nuclear genes, including *Raa1*, *Raa2* and *Raa3*, which we have isolated and characterized. These factors are part of large ribonucleoprotein complexes in the chloroplast.

4.2.2. Small genomes in plastids from primary and secondary endosymbioses

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Primary plastids, which are found in green algae, land plants, red algae and glaucocystophytes, are surrounded by a double membrane and evolved by the incorporation of a cyanobacterium-like cell and its reduction to a plastid. Other plastids, the complex plastids, originated from secondary endosymbiosis. Here, a eukaryotic phototroph with primary plastids was engulfed and reduced to a plastid surrounded by three or four membranes. Some secondary evolved algae show highly reduced genomes. I will present new findings on dinoflagellates and cryptomonads, and how these organisms compartmentalize the genetic information. Neither in primary nor in secondary endosymbiosis, organisms are known, which are related to the phototrophic symbiont. This situation complicates the reconstruction of genomic evolution in early steps of intracellular co-evolution. However, several intracellular cyanobacteria are known, which can be used as model systems for the transition of free-living organisms to permanent intracellular symbionts. One of these transitions will be presented in the case of a cyanobacterium/diatom interaction.

4.2.3. Co-evolution of nucleus and plastid - the model *Oenothera*

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The evolution of eukaryotic genomes, which in plants traces back to endosymbiotic conglomerates of three cells, was coined by three basic processes that changed the genetic potentials of the symbiotic cells into a single integrated compartmentalised genetic system. *Compartmental co-evolution*, one of these processes, becomes apparent after organelle exchanges between species which can lead to serious developmental disturbances in the resulting genome-plastome hybrids. - The genus *Oenothera* represents a unique resource to study the impact of nucleoplasmic interaction in plant development and evolution, due to a favourable combination of genetic features and the existence of a highly developed cytogenetics and taxonomy of genome, plastome, and chondriome. This allows to exchange plastids and nuclei, also to substitute individual chromosome pairs between species. - We have characterised in natural and artificial combination the five basic plastid chromosomes and six basic genomes of subsection *Euoenothera* to study the interspecific plastid/nuclear network in developmental and speciation processes that is possible in a continental dimension.

4.2.4. Transcription in plastids: ontogenetic and phylogenetic aspects

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The highly complex transcriptional machinery of plastids provides fascinating insights into the ways leading to the integration of an organelle into the genetic system of a eukaryotic cell. Plastid genes are transcribed by a plastid-gene encoded RNA polymerase (PEP) and, at least in land plants, by one or more nuclear-gene encoded RNA polymerase(s) (NEP). PEP is a bacterial-type RNA polymerase such as already used by the cyanobacterial ancestors of plastids. Several data indicate that NEP is represented by one or more phage-type RNA polymerases. The nuclear gene for NEP originated by duplication from the gene coding for the mitochondrial RNA polymerase in nearly all eukaryotes. PEP promoters are found upstream of genes for proteins needed in photosynthesis and of many genes with house-keeping function. The nucleus controls PEP activity by supplying various sigma factors and by NEP needed to transcribe the genes for PEP subunits. NEP gene transcripts and transcripts of NEP-transcribed genes show eminently different levels in dependence on age and type of tissue indicating an individual and spatial developmental role.

4.2.5. Sequences affecting translation of plastid mRNAs

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The plastid translation system includes bacterial-like ribosomes and tRNAs. However, about two-thirds of tobacco plastid mRNAs contain no Shine-Dalgarno-like sequence (typically GGAGG) at a proper position in the 5' UTR. To study plastid translation, we previously developed an *in vitro* translation system from tobacco chloroplasts. Here, we improved it by using a gene coding for green fluorescent protein and by modifying extraction and reaction conditions. The improved method is ca. 100-fold more active than the original system, extremely lower in background and requires no facility for radioactive materials. Using this system, we identified *cis*-sequences and repressing sequences of several plastid mRNAs, and analyzed effects of codon usages and of codons immediately downstream of AUG.

4.2.6. Assembly-controlled regulation of chloroplast gene translation

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Studies of the biogenesis of the photosynthetic protein complexes in the green algae *Chlamydomonas reinhardtii* have stressed the importance of the concerted expression of nuclear and chloroplast genomes. The accumulation of chloroplast and nuclear encoded subunits from photosynthetic complexes is a concerted process. Many unassembled subunits are rapidly degraded but others show an assembly-dependant regulation of their rates of synthesis, known as a 'Control by Epistasy of Synthesis' or CES process. For example, the rate of synthesis of cytochrome *f*, a major subunit of the *cyt. b6f* complex encoded by the chloroplast *petA* gene, is decreased 10 fold in the absence of its assembly partner SUIV. This results from an autoregulation of *petA* mRNA translation that involves negative feedback from the C-terminal domain of the unassembled polypeptide. Our results show that assembly-dependent autoregulation of translation initiation are widely spread in the chloroplast of *C. reinhardtii*, since all major photosynthetic complexes contain at least one CES subunit. These regulations would involve nucleus-encoded gene specific translation activators.

4.2.7. Assembly of membrane integral quantum traps

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Through evolution, membrane integral protein complexes are used to trap light quanta for photosynthesis. Protein complexes of the photosynthetic machinery have been crystallized; however, the position of several subunits within the complexes is not clear and assembly of the subunits is a just emerging field of interest. Here, we show that *Synechocystis* PCC6803, *Chlamydomonas reinhardtii* and higher plant chloroplasts provide excellent model

systems to study the assembly process. In this direction we address the regulation of the assembly process and present components that participate in the molecular regulation of the assembly process. Technically, we present that assembly of membrane protein subunits into functional complexes can be well studied by using 2 BN-SDS-PAGE and demonstrate the detection of protein complexes by fluorescence and the identification of membrane integral alpha-helices by mass spectrometry.

4.3.1. The control of apical cell growth and morphogenesis by ion dynamics

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Despite copious amounts of detailed info at the physiological and, more recently, on the molecular side, regulation of growth in pollen tubes still lacks an integrative model. In the past we have proposed that "ion dynamics" maybe a common denominator in the regulation of living cells. In pollen tubes, fluxes are polarized, implied that carriers for protons, calcium, chloride and potassium shows non-linear patterns in space (polarized distribution of carriers) and also in time (oscillatory and chaotic behaviors). In accordance, P-ATPases are excluded from the apex membrane by a mechanism that involves polarized translation, actin cytoskeleton and membrane recycling. We are exploring transcriptomic information out of Arabidopsis genechip to boost these kinds of correlations and develop functional tests for specific molecular entities. Yet, data emanating from the different fields must be integrated in a coherent theoretical way. A simple computer model balance the intracellular turgor with differential rigidity of the cell permits the exact simulation of most of the basic cellular features of pollen tubes.

4.3.2. Cell wall architecture and anisotropic growth in pollen tubes

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The cell wall is one of the structural key players regulating pollen tube growth, since plant cell expansion depends on an interplay between intracellular driving forces and the controlled yielding of the cell wall. Since pollen tubes show extreme anisotropic growth behavior, the physical properties of the cell wall should be expected to differ between growing and non-growing regions. We evidenced these differences using local deformation performed by micro-indentation. This approach revealed a strong gradient in rigidity along the longitudinal axis of the cell, that cannot be explained by geometrical features as demonstrated with finite element modeling. To identify the roles of individual cell wall components for cellular architecture we used enzymes that specifically digest or change the configuration of cell wall polymers such as callose, cellulose or pectins. We related the mechanical data with the distribution of these polymers assessed with fluorescence labeling techniques. This revealed that in particular the distribution of methyl-esterified and acidic pectins seems to be a fundamental structural feature of anisotropic cellular growth.

4.3.3. Wall structure and oscillating growth of *Nicotiana tabacum* pollen tubes

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Using cryo-FESEM, we assessed porosity and 3-D construction of pollen tube walls. Below phreatic point-sublimation of water from broken pollen tube walls revealed a network of continuous fibers interconnected by short rods with 50nm spacing. Such a structure cannot form a mechanical barrier even for larger molecules, such as proteins. Using the JIM 5 antibody or the basic dye Toluidine blue, a clear, banded pattern was seen in the wall. Using the 2F4 antibody for egg-box pectin, similar patterns were detected. Remarkably, the same pattern was found in the much later deposited secondary wall after Aniline blue staining for callose. Progressive removal of water soluble wall components with EDTA showed the tube cell to be tiled with scales that probably represent

the bands seen after Toluidine blue staining. In contrast to the secondary wall, the primary wall almost completely dissolves with EDTA-extraction, indicating its coherence to depend on pectin. Banding patterns of pollen tubes grown *in vitro* appeared to depend on the media used, on growth conditions, but also on culture time. *In vivo*, banded patterns occur less frequent, but may appear regularly after stress.

4.3.4. Pollen tube growth and guidance: $[Ca^{2+}]_c$ is a key element in the crosstalk of signaling pathways which modulate apical secretion

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Pollen tube growth relies on an extremely fast delivery of new membrane and wall material to the apical region where growth takes place. Despite the obvious meaning of this fact, the mechanisms that control this process remain very much unknown. A tip-focused $[Ca^{2+}]_c$ gradient is known to play a central role in the regulation of pollen tube growth and modulation of the $[Ca^{2+}]_c$ concentration results in changes in the rate and direction of growth. One of the targets of this Ca^{2+} signalling pathway was therefore claimed to be the secretory pathway and we have been studying this process. We found that different signaling pathways (from phospholipids to calmodulin, from cAMP to phosphoinositides) converge in the modulation of apical secretion. Interestingly, the common link among the different signaling pathways is their ability to modify Ca^{2+} levels thus pointing to the key role of this ion in the crosstalk. A working model of how this can be achieved is discussed.

4.3.5. Variability of the actin staining pattern in pollen tubes

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We investigated the cytoskeleton of pollen tubes using an improved method for actin visualization, the simultaneous fixation and staining with fluorescent phalloidin. In all pollen tubes, longitudinal actin filament bundles were present in the shank and the subapex contained more randomly oriented bundles. The actin cytoskeleton of the tip consisted either of a meshwork of randomly oriented actin strands or of longitudinally aligned actin bundles. The longitudinal bundles had a length of about 10 μ m and formed a dome-like structure immediately behind the tip. In pollen tubes with apical actin meshworks mitochondria moved up to the subapex before joining the grainwards directed cytoplasmic streaming. The mitochondria of pollen tubes containing apical actin bundles migrated up to the tip. These observations and data obtained by video-enhanced contrast microscopy indicate that fine-meshed actin networks form a barrier for the movement of large organelles (but not vesicles) towards the tip. Growth rates were similar for all pollen tubes suggesting that the orientation of apical actin filaments has no significant effect on the deposition of cell wall material.

4.3.6. Making and steering the cell polarity engine in pollen

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Male gametophytes of Spermatophytes are tiny organisms specialised in dry conditions survival and sexual reproduction. Siphonogamy evolved from a weeks-consuming process in some Gymnosperms to the fertilization race taking just a few hours in Angiosperms. Directionality and speed of growth are crucial parameters. Using systems of *in vitro* pollen tube cultures we are characterizing selected aspects of pollen development, especially in respect to processes potentially regulated by small GTPases of Rab and Rho families. In particular, we focus on the final steps of exocytosis, involving the exocyst complex, phospholipases D activities, and the function of formins. We have observed specific synthetic/preparatory phase before tobacco pollen tube emergence which results in the establishment of self-perpetuating tip-focused secretory machinery. We will report on data providing evidence that there are positive-feed back processes operating at the tip which are responsible for the sustained directional growth

resulting eventually in a successful fertilization. Supported by GAAV CR A6038410, GACR 204/05/0268 and EU-HPRN-CT-2002-00265 TIPNET grants.

4.3.7. Pollen tube growth mutations in *Arabidopsis*

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Pollen tube growth inside the pistil is a key step of sexual plant reproduction, allowing the traveling male gametes to reach the waiting female oosphere and central cell. Upon hydration on the stigma, the pollen grain containing both the sperm cells germinates and elongates a tube through tip growth in the female tissues, that will target an ovule where to deliver its content. Cell biology of pollen tube polar growth is well described for a number of species, and many of the key actors have been characterized. However, the genetic control for this tip growth process remains unravelled. The development of *Arabidopsis* model and the increasing number of available mutant lines of this specie offer the possibility to screen for mutations leading to pollen tube growth defect, and potentially identify genes determinant for this process. We isolated two novel genes, *KINKY POLLEN* and *POKY POLLEN TUBE*, encoding proteins that are essential for normal pollen tube tip growth. The detailed study of both genes suggests that their roles might not be restricted to pollen tube elongation, but would also concern other polar growth processes.

4.4.1. The Early Evolution of Water-Conducting Cells in Vascular Plants: Integration of Paleobotanical and Neobotanical Data

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Although there is clear evidence for the establishment of terrestrial plant life by the end of the Ordovician, the fossil record indicates that land plants remained extremely small and structurally simple until the Late Silurian. Among the potential key innovations associated with this first major radiation of land plants is the evolution of tracheids, water-conducting cells defined by the presence of lignified secondary cell wall thickenings. Comparative studies of key basal (and potentially plesiomorphic) extant vascular plants, along with structural and chemical analyses of early fossil tracheophytes, are slowly beginning to yield formal hypotheses for the developmental evolution associated with establishment and early diversification of tracheids. Finally, the issue of the potential homology (or lack thereof) of tracheids with the hydroids of *Aglaophyton* and bryophyte grade plants will be discussed.

4.4.2. Diversification of the secondary xylem structure in early lignophytes

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The middle Devonian aneurophytealean progymnosperms are generally accepted candidates as ancestors of the earliest seed plants. Their wood is composed of rather broad tracheids with crowded bordered pits on all walls and homocellular, tall and narrow rays. Suggested evolutionary trends include: 1) a decrease in diameter of tracheids (in both arborescent archaeopteridaleans progymnosperms and pityans seed plants), 2) inversely an increase in diameter of tracheids in the other early seed plants; 3) a decrease in pit seriation in archaeopteridalean, protopityans and some early seed plants; 4) a restriction of pitting to the radial wall of tracheids in early seed plants; 5) an increase in mean width of rays in archaeopteridaleans and seed plants. Some characters are rare (ray tracheids, xylem parenchyma) or so variable in the ancestral group (ray height) that their polarity is difficult to establish. To summarize, the early lignophytes, known from the Mid Devonian to the Early Carboniferous exhibit successive, apparently independent, trends in their wood structure representing as many developmental adaptations to different habits and environments.

4.4.3. Morphospaces and exploration of evolutionary phenomena in stems

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We construct hypothetical two-parameter graphs as simple depictions of the "big picture" of the evolutionary biology of stems.

We use these “morphospaces” to direct searches for undocumented anatomical and biomechanical phenomena, and provide examples from our recent research. The axes of these morphospaces reflect common parameters such as stem construction and mechanical or conductive behavior. E.g. flexural stiffness (EI) of early ontogenetic stages on Y axis, EI of late stages on X axis. We discuss the allometry, anatomy, and biomechanical behavior of the western Mexican dry tropical forest endemic *Bursera instabilis*, an apparent example from the upper left corner of this morphospace (self-supporting when young, branches non self-supporting with age), along with comparisons of the other members of the *simaruba* clade of *Bursera* (Burseraceae). We also discuss the role of the mechanisms that permit “movement” from one area of morphospace to another (with an example of heterochrony in Moringaceae), and the forces that might be favoring such changes.

4.4.4. Homoplasy in the major trends of xylem evolution

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Once the major “Baileyian” trends in xylem evolution were established early in the last century, the apparently irreversible nature of character state transitions made systematic wood anatomy highly respected as a way of confirming or contradicting the phylogenetic position of woody plant groups. Recent insights in plant phylogeny combined with progress in understanding water transport processes in wood, have led to a more homoplasious and dynamic view of xylem evolution. There is a clear need to re-evaluate long established concepts of phylogenetic wood anatomy, which remain hampered by problems of typology and ambiguous evolutionary frameworks. More correlation between functional and comparative studies would open new ways to amplify observations of xylem physiologists by comparative inference. Future research should emphasise investigation of the genes involved in the control of key xylem characters, especially the development of perforation plates and bordered pits.

4.4.5. Inter- and intraspecific variations in the structure of intervascular and interfiber pit membranes, as observed by field-emission scanning electron microscopy

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Information about structural variations in the pits of angiosperm remains limited and fragmentary for a large number of species and types of pit. Thus, we have studied the structure of intervascular and interfiber pit membranes in some dicotyledonous wood by field-emission scanning electron microscopy. The intervascular pit membranes of some species consisted of thin surface layers and a dense middle layer, while no similar middle layer was detectable in the other species. In the latter type, microfibrils of some of the pit membranes were sparsely interwoven in small areas of the pit membranes and openings of up to several hundred nanometers in diameter were present in such regions. Interfiber pit membranes tended to be porous with the specialization of fiber. Pit membranes between fibers with distinctly bordered larger pits were dense, and consisted of a few layers. By contrast, pit membranes between fibers with minutely bordered smaller pits were usually porous, and layered structure was not apparent. Our observations suggest that the structure of intervascular and interfiber pit membranes is more complicated than has previously been acknowledged.

4.4.6. Efficiency vs safety trade-offs underlying xylem structure and function

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Air-seeding probably occurs when air is pulled through the largest pore of pit membranes into a water-filled xylem conduit. It leads to embolized conduits and thereby reduces hydraulic and stomatal conductance. The fact that xylem is usually not more resistant than required in a specific habitat suggests that there are costs involved in developing cavitation-resistant xylem. Cohesion-driven transport requires an adequate reinforcement of conduit walls. The more negative the pressure can become in a conduit, the greater are the potential forces acting on its walls. Besides this mechanical cost of developing safe xylem, there is a hydraulic cost. We found that resistant vessels have less pit membrane area than vulnerable

ones. This suggests that cavitation is caused by the rare largest membrane pore, whose average size increases with total pit area per vessel. Total pit area was correlated with vessel surface area. As a result, we observed a trade-off between vessel resistivity and cavitation resistance. The constraint on pit area and vessel surface area explains why vessels are not longer, and why pits account for c. 50% of the total xylem resistivity.

4.4.7. Embolism repair: a functional perspective

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Stems are complex structures charged with the task of transporting water under substantial negative pressure that often leads to cavitation. The mechanism by which some plants are able to reverse cavitation and restore water flow through embolized vessels remains poorly understood. Although the process requires energy input and thus the involvement of living cells, it remains unknown how plants are able to increase the hydrostatic pressure within cavitated conduits without raising the pressure of the entire vascular system. Based on studies of *Gossypium* sp. (cotton) and *Acer rubrum* (red maple), we present data relevant to the driving force for water flow into cavitated conduits and address the time scale for such repair using in-vivo MRI observations of stems in transpiring plants. How these data help to distinguish between proposed mechanisms for embolism repair will be discussed in relation to xylem structure and function.

4.5.1. Modern evidence in regard of the S distyly supergene linkage group in *Primula*.

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Of 430 *Primula* species, 91% are distylos with reciprocally positioned anthers. Long-styled (‘pin’) individuals (ss) have smaller, more numerous pollen and longer stigma papillae than short-styled (‘thrum’) plants (Ss) and distyly is linked to a diallelic sporophytic incompatibility. True-breeding thrums are unknown, suggesting a thrum-linked recessive lethal. Most homostyles have a recombinational origin allowing earlier workers to propose a distyly supergene linkage group with three recombinable elements. It is now known that female recognition is independent of style and papilla length, and male recognition is independent of, and can be recombined with, the locus controlling pollen size. Dominance control for male mating type is independent of that for pollen size. Incompatibility recognition is located in at least two sites in the gynoeceum, and differs between pin and thrum selfs. Male and female expressed recognition factors can be recombined, suggesting that the gene is duplicated in association with different operators. Eight recombinable elements may occur in the supergene, which may have evolved more than once within the genus.

4.5.2. Reconstructing the history of heterostyly in *Primula* and embedded genera with a 250-taxa cpDNA phylogeny

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Since Charles Darwin’s work on distyly in *Primula* (ca. 430 spp.), the genus has played a central role in our understanding of distyly’s function and genetic control. In distylos species, populations maintain a genetic polymorphism that produces two flower morphs, each differing reciprocally in the position of their anthers and stigmas. We generated a 250-taxa cpDNA phylogeny with data from the matK gene, rpl16 intron, trnL/F spacer, and trnL intron for *Primula* and embedded genera (*Dodecatheon*, *Cortusa*, *Dionysia*, and *Sredinskya*) and reconstructed the history of the dimorphism and the correlated self-/intra-morph physiological incompatibility. We offer evidence that distyly arose prior to the most recent common ancestor of all extant taxa of *Primula* and that none of the 35 monomorphic taxa of *Primula* diverged prior to the origin of distyly. We consider the ramifications of this for models of the evolution of distyly that rely upon the existence of primitively monomorphic species of *Primula* (i.e., the existence of “primary homostyles”).

4.5.3. Phylogenetic relationships of the heterostylous *Vitaliana primulifera* (Primulaceae)

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Although Primulaceae are a textbook example for heterostyly, its occurrence outside the largest genus *Primula* is rare. One such case is the monotypic European endemic genus *Vitaliana*, which has been separated from the homostylous genus *Androsace* mainly because of the presence of heterostyly. Previous molecular studies using nuclear ITS and plastid trnLF sequences, however, show that *Vitaliana* nests within the European *Androsace* sect. *Aretia*. A close relationship of these two groups has already been suggested by certain morphological traits and karyological data. We extended the sampling to include both more sequence data (matK, rpl16) and more accessions of *Vitaliana* to (i) pinpoint the closest relative of *Vitaliana* among the homostylous species of *Androsace* sect. *Aretia*, and (ii) to address the phylogenetic relationships of the mostly allopatric intraspecific taxa within *Vitaliana*, traditionally recognized on the subspecific level.

4.5.4. Molecular genetic analysis of the S locus in *Primula vulgaris*

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Despite intense and historical interest in the ecology, evolutionary biology and classical genetics of floral heteromorphy in *Primula vulgaris*, almost nothing is known about the molecular basis of heteromorphic flower development. In order to investigate the biological processes underpinning this classical breeding system, we have applied molecular genetic approaches to identify genes and DNA sequences located at the *Primula* S locus. These studies have identified several sequences that are tightly linked to the S locus and as such provide the first molecular markers for this complex locus. We have also used classical and molecular genetic analyses to study mutant phenotypes that show linkage to the S locus. In combination, the analysis of available mutant phenotypes, S locus-linked DNA sequences and recombinants has enabled us to generate a map encompassing the S locus. This map includes genes that flank the locus. These sequences define the boundaries of the locus and will facilitate the identification and ordering of BAC clones across this region. Recent progress towards the molecular characterisation of the S locus and associated genes will be presented.

4.5.5. Genetics and evolution of breeding systems in *Turnera* (Turneraceae): Using the Primulaceae as a paradigm

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Primula provides a valuable model for investigations of distyly. We use this model as a basis for comparison of the genetics and evolution of breeding systems in *Turnera*. Distyly is widespread in the Turneraceae and polyploidy and karyotype evolution have played significant roles in speciation in this genus. Distyly tends to breakdown to self-compatible homostyly in high polyploids, but this pattern is not absolute and may be restricted to few clades. Distyly is determined by a single Mendelian locus with two alleles. Short-styled plants are Ss while long-styled plants are ss. Homostylous species and mutants appear to be determined by an allele, S^H , at the distyly locus with the dominance relationship $S > S^H > s$. The inheritance and compatibility relationships among long-styled, short-styled, and homostylous plants, are consistent with the possibility that a linked gene complex determines distyly. Studies of the molecular basis of distyly in *Turnera* have revealed two proteins that localize to the transmitting tissue of short styles. Genetic mapping to localize distyly has revealed two genes that co-segregate with the distyly locus.

4.5.6. Ecological contexts for reproductive shifts in *Primula* to secondary homostyly

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The complex genetic, morphological and biochemical foundations of heterostyly have been studied with a particular focus on *Primula*. In secondary homostyly, a crossover within the genetic

linkage enables self and intra-morph fertilization, in contrast to the obligate outcrossing enforced in the heterostylous condition. Homostyles can be rare mutants in normally heterostylous species or separate taxa with distinctive morphologies and distributions. Three models for homostyle success are presented: the diploid reproductive assurance model, the hybrid opportunist model, and the allopolyploid secondary contact model. They share ecological contexts enhanced by climatic and anthropogenic perturbation such as unreliable pollination, population fragmentation, and proximity of novel habitats or migration routes. Although successful establishment of homostyles is supported by selfing potential, some taxa show herkogamous traits that may enhance or maintain outcrossing opportunities.

4.5.7. Evolution of breeding systems and ploidy levels in *Primula* sect. *Aleuritia*, a paradigm of the secondary contact model

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Primula sect. *Aleuritia* displays a fascinating array of breeding systems, ploidy levels, and biogeographic patterns. The observed correlation between polyploidy, homostyly, and arctic distribution has been explained by the secondary contact model. Glacial advancement during the Pleistocene caused fragmentation of diploid, heterostylous populations. As glaciers retreated, the differentiated diploid populations came into contact again and hybridized, giving origin to polyploid, homostylous taxa. To investigate this proposed model and test specific hypotheses on the origins of polyploids, we generated detailed phylogenies of *Primula* sect. *Aleuritia*, using one nuclear and five chloroplast DNA regions. In the resulting trees, the polyploid taxa did not form a monophyletic group, but originated independently from within different diploid clades, in agreement with the secondary contact model. Furthermore, our results supported conflicting relationships of most polyploids in the nuclear and chloroplast trees, as expected under the hypothesis of hybrid origins.

4.6.1. Adaptive structures of fungi as a key to the interpretation of their evolution and mechanisms of ecological and geographical distribution

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An approach is suggested according to which all systematic criteria are regarded as ecologically inert or ecologically active. The first ones are accepted as sure markers of belonging to any phylogenetic line. Ecologically active characters are determined by pattern genes and were shaped under the influence of fungus adaptation to the environment, substrate and a strict ecological niche. Winning of the space, assimilation of the new substrates, increase of competitive ability and the achievement of the best conditions for the formation and distribution of spores are the main stimuli in evolution of the adaptive structures. Phenomenon of parallel lines of development and convergence must be taken into consideration when regarding the problem. Nutritional functions of mycelium and morphological structures for spore distribution are regarded. Types of adaptive evolution in the different groups of fungi are discussed.

4.6.2. Agaricales (Basidiomycota - Fungi) species in Brazil

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The Agaricales (Basidiomycota - Fungi) are poorly known from Brazil, despite this country as been called as having one of the greatest biodiversity in the world. The first works were carried out by European botanists but the most important work, as been that of Johannes Rick, who studied Agaricales (among other fungi) from Southern Brazil, providing description of almost 700 species and being called now as the father of Brazilian mycology. Studying collections done in several Brazilian states since 1986 and reviewing the bibliographical data and herbaria collections, a list of the species known up to now is provided together with descriptions and illustrations to be employed in the first book on Agaricales for this country (in preparation). The data collected indicates that there are 1230 species and 137 genera known to the area. The description of 12 species new to science is provided and the new

occurrence of other 16 species of Agaricales is indicated. Tricholomataceae is the family with greater number of species, followed by Agaricaceae and Cortinariaceae. The agaricobiota from neighboring countries is compared and the relationships are discussed.

4.6.3. The nordic saproxylic network

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The Nordic saproxylic database has the ambitious aim to organize species-specific ecological information for all wood-inhabiting species in the Nordic region (i.e. Denmark, Finland, Norway and Sweden). One major organism group is fungi of which both Ascomycetes and Basidiomycetes are included. The other major groups are invertebrates, vertebrates, mosses and lichens. By the end of 2004, the number of species in the database is about 4000. We expect the number to approach about 7000 species during the coming years. The species-specific ecological information categorizes the association of a species to different wood qualities (decay, diameter, microhabitat, etc.). Each species is ranked according to their preferences for such host characters. The species associated to dead wood form a food web where many species have ecological relationships at higher trophical levels (through predation, parasitism, symbiosis, etc). Such species are also included in the database. The database includes information about which species that is recorded in each country and from which country the ecological data origins.

4.6.4. Molecular diversity of ectomycorrhizal and associated fungi in pine forest on serpentine soil

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The diversity and phylogenetic composition of ectomycorrhizal and ectomycorrhiza-associated fungi in a natural forest on serpentine was explored. 14 Ectomycorrhizal fungi associated with *Pinus sylvestris* growing on serpentine outcrops were identified, aphyllophorean fungi and Russulaceae being dominant. Ectomycorrhiza associated fungi were assessed by cultivation and by molecular cloning, RFLP typing and sequence analysis. Species spectra of ectomycorrhiza-associated fungi assessed by cultivation and by molecular cloning were almost entirely different, the only fungus found by both methods being *Phialocephala fortinii*. Among the fungal clones, ascomycetes with helotialean, dothideomycetalean chaetothromycetalean and with hypocrealean affinities were present. One clone belongs to a new fungal lineage with closest affinities to Taphrinomycetes and Orbiliomycetes (Ascomycota). The results of a screening of ectomycorrhizal samples with newly designed specific primers for mycorrhiza-associated fungi suggest that the fungal community in the ectomycorrhizosphere is structured by taxon-specific interfungal interactions.

4.6.5. Micro-fungi on rock and man-made materials - conquerors of an extreme environment

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Micro-fungi are commonly regarded as inhabitants of soil and decomposers of organic materials in nutrient rich environments. However, fungi are also found on inorganic substrata and live under oligotrophic and poikilotrophic conditions. Material surfaces including natural and man-made materials represent an ecological niche characterized by low aw, high UV-radiation and high temperatures. In temperate climatic regions the fungal community on rock and material surfaces is dominated by fungi belonging to the common soil flora, in semi arid and arid regions those fungi are unable to survive and the rock fungal community is shifting towards darkly pigmented microcolonial fungi. In soil, however, these slow-growing fungi are out-competed by rapidly growing moulds. Due to a very similar in-situ morphology and a lack of differentiation the species diversity of microcolonial fungi was believed to be relatively low. Molecular data, however, show that

there is high diversity and the number of newly described species is constantly growing.

4.6.6. Occurrence of filamentous microfungi and effects of nutrient, pH, temperature and salinity on growth of selected antarctic soil fungi from Windmill Islands

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A mycological survey was conducted during summer of 2002, 2003 and 2004 on soil samples at Windmill Island, Antarctic continent. Soil plated method was used in this study and were incubated at 4°C. A total of 220 of soil samples were collected and these comprised of 245 strains of fungi representing 10 species of soil microfungi. These include *Antarctomyces* sp., *Aureobasidium*-like sp., *Cadophora malorum*, *Geomyces* c.f. *cretaceous*, *Mucor* sp., *Thelebolus* sp., *Trichosporiella* c.f. *cerebriformis*, 1 unidentified Ascomycete sp. and 2 unidentified species. Studies on the effects of nutrient, pH, temperature and salt concentration on fungal growth were carried out on selected Antarctic soil fungi. *Phoma* sp., *Aureobasidium* sp., *Thelebolus* sp. and *Antarctomyces* sp. were cultured and growth rates were compared on two media, Potato Dextrose Agar and Corn Meal Agar with different salinity, pH and temperature. The plates were incubated at 4 and 25 °C and measurements of the colony diameter were recorded. Ultrastructure studies were carried out and occurrence of the microfungi were compared with other part of the Antarctic to discuss their mycogeographic distribution.

4.7.1. Size, distribution and phytogeographical position of the Balkan endemic flora

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The project "Mapping the endemic flora of the Balkans" began in 2002. It is estimated that c. 30% (2600-2700 species and subspecies) of the total Balkan flora is endemic. 2000 taxa have been mapped, representing c. 75% of the flora; this is adequate for reliable floristic and phytogeographical analysis. The distribution is mapped using 50 x 50 km UTM squares. The maps are presented for genera and families as well as summarized for c. 2000 taxa. The type of distribution and centres of diversity for large and important groups are provided. Numerous squares (regions) have extremely high numbers of endemics and several squares have more than 100 mountain endemics. Numerous endemics are restricted to only one or two adjacent squares. Similarity indices of particular squares clearly demonstrate floristic correlations in certain Balkan regions. For each endemic taxon, the distribution of its closest relative(s) from a taxonomic section or series is provided thus indicating floristic relationships and affinities between the endemic Balkan flora and that of the surrounding regions, e.g., Turkey, Caucasus, Alps, Iberian and Apennine peninsulas and the Mediterranean.

4.7.2. Differentiation of Turk's cap lilies in Balkan region: molecular-cytogenetic approach

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Taxonomical status of the Balkan endemic Turk's cap lilies (*Lilium albanicum*, *L. bosniacum*, *L. jankae*, and *L. chalcidonicum*) is still remained unresolved. The most of these taxa are frequently included in *Lilium carniolicum* complex. We report the first data demonstrating molecular-cytogenetic differentiation among these closely related lilies. In this comparative study of four endemic taxa with *L. carniolicum*, we used fluorochrome banding for detection of GC and AT rich DNA regions, fluorescence *in situ* hybridization (FISH) to determine the number and position of 18S-5.8S-26S and 5S rDNA loci, silver staining to study NORs activity and DAPI after FISH experiment that revealed the number and position of heterochromatic bands. The genome size of these taxa, estimated by flow cytometry, were also considered. The results based on

molecular-cytogenetic markers allowed distinction among these lilies on nuclear and chromosomal level and revealed a clear taxonomical differentiation within this endemic group.

4.7.3. The evolution of the Balkan flora with particular reference to Greece

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The Balkan flora is predominantly of temperate origin and has Central European and Anatolian affinities. Tertiary relicts of tropical affinities include a few mountain endemics such as *Jankaea heldreichii* and some widespread woody lowland species such as *Cercis siliquastrum* and *Ficus carica*. The degree of endemism increases in a southerly direction and with increasing latitude, being highest in the mountains of Crete. The endemics are predominantly diploid. Edaphic endemism is common especially on serpentine substrate which is widespread in the mountains of NW Greece and occurs intermittently through Albania to Bosnia. The Aegean islands, which are remnants of a broad land bridge once connecting Greece with Asia Minor, have a somewhat impoverished flora. The phytogeographical subdivision of the Aegean area originally proposed by Rechinger has been largely corroborated by experimental studies of differentiation in certain species groups and also by statistical analyses of the whole flora. The Flora Hellenica Database currently holds 620.000 records and is becoming a powerful tool in biogeographical analyses.

4.7.4. The Bulgarian flora - present state and future challenges

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Intensive botanical exploration of the Bulgarian flora began in the late 19th century. The first Bulgarian flora, "Flora Bulgarica" by J. Velenovský included 2542 species and was published in 1891. This flora stimulated many Bulgarian botanists to contribute the results of their researches towards a more comprehensive, two-volume publication in 1924-25, "Flora na Balcaria" by N. Stojanov and B. Stefanov. The work enumerated 2936 species. Three later editions were issued, in 1933 (with 2957 species), 1948 (3089 species) and 1966-67 (3072 species). The first volume of a projected 12-volume, modern, critical and illustrated work, "Flora Reipublicae Popularis Bulgaricae" was published in 1963. Ten volumes have been published so far and the project continues. The flora of Bulgaria is currently estimated to comprise c. 3900 species of vascular plants. The taxonomic composition and endemism are briefly considered and the distribution of species in the different floristic regions. Contemporary major projects are presented. The problems and challenges facing the writing of the modern Flora of Bulgaria are discussed in the context of a broader Balkan perspective.

4.7.5. The distribution of Balkan endemics related to the Central European mountain element

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It is estimated there are 2600-2700 endemic species and subspecies in the Balkans. The origin and phylogeny of each is of particular interest. Most Balkan endemics have Mediterranean and oro-Mediterranean relatives. A small number (c. 200 taxa or 7% of the total) have distinct connections with the mountain flora of temperate Central Europe, from the Pyrenees across the Alps, N Apennines towards the Carpathians and to the Arctic. According to size and range of distribution, the related taxa can be classified as widespread (Pyrenees to Carpathians), or narrow (restricted to one mountain massif). The distribution of the endemic Balkan vicariants is mapped using 50 x 50 km UTM squares; the majority occur in the high mountains of W and C Balkans and indicate floristic links to the Alps and Carpathians, much less so to the Pyrenees. Two main groups of Central European mountain flora can be distinguished, viz., the well-differentiated and distinct vicariant species which indicate early, Tertiary connections between the Balkans and Central European mountains, and the geographical races or subspecies which indicate more recent differentiation during the Ice Age.

4.7.6. Systematics and phylogeny of *Eryngium* L. (Apiaceae, Saniculoideae)

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A complete revision of *Eryngium*, comprising ca. 250 species, is presented for the first time since more than 90 years. This new revision (Wörz) is based mainly on morphological and ecological characters and chromosome numbers. A new subgeneric and sectional classification based mainly on morphological characters is presented. This classification is compared with a molecular analysis (ITS, rps16) of *Eryngium* and relatives (Schmalz/Kadereit), which partly supports the classification and allows some conclusions on inter- and intrageneric relationships: 1) *Eryngium* may be paraphyletic in relation to *Sanicula*, *Hacquetia*, and *Petagnia*. 2) The two major subgroups of *Eryngium* are the Old World subg. *Eryngium* and the (largely) New World (plus Australia) subg. *Foetida*, *Fruticosa*, *Monocotylodea* and *Semiaquatica*. These two clades are supported by the structure of basal leaves. These are palmate in subg. *Eryngium* (plus *Sanicula*, *Hacquetia*, *Petagnia*) but pinnate or monocotyledonoid in the remainder of *Eryngium*. In New World *Eryngium*, the basalmost lineages (subg. *Semiaquatica*) are distributed in Europe. This may suggest an Old World origin of New World *Eryngium*.

4.7.7. Evolution and taxonomy of *Veronica* in the Balkan Peninsula

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With more than 6500 species of native seed plants on the Balkans and almost a third of them endemic, the Balkan Peninsula is known to be a place for diversification and formation of new species and an important source for colonization after the ice ages. One plant group, which is very species-rich on the Balkan Peninsula, is the genus *Veronica* (Plantaginaceae s. l.). Our current estimate for species diversity of the genus in Europe shows that 55 of 70 species in Europe occur on the Balkan Peninsula with 20 of them (sub-)endemic there. This shows the importance of the region for speciation in *Veronica*. Based on their distribution and that of their relatives they can be grouped in alpine, Mediterranean, eastern Steppe, Anatolian, introduced and in-situ elements. Detailed DNA-based systematic and phylogeographical analyses in *Veronica* help further demonstrate origins, patterns of diversification and centers of genetic diversity within the Balkan Peninsula for those elements. Phylogeographical analyses of two alpine species (*V. alpina*, *V. bellidioides*) and the *V. spicata*-complex (*V. sect. Pseudolysimachium*) are presented in more detail.

4.8.1. Systematics of Euphorbiaceae s.l. lineages

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Following APG II, Euphorbiaceae s.l. have been split into five families that remain more or less closely related as members of Malpighiales. Molecular data from 2-5 genes for each lineage have allowed the construction of robust generic-level phylogenetic trees that depict new relationships and necessitate revised classifications. With reference to prior s.l. classifications: Pandaceae include Acalyphoideae-Galearieae, Phyllanthaceae include Phyllanthoideae (with *Croizatia* and *Dicoelia* but excluding *Centropilacus* and *Drypeteae*), Picrodendraceae include Oldfieldioideae (except *Croizatia* and *Paradrypetes*), Putranjivaceae include Phyllanthoideae-Drypeteae (excluding *Lingelsheimia*), and Euphorbiaceae s.s. include the remaining uniovulate euphorbs. A recent 8-gene analysis shows Phyllanthaceae and Picrodendraceae to be well-supported sister taxa. Intrafamilially, Phyllanthaceae and Euphorbiaceae s.s. are least concordant with prior groupings. Non-molecular data support new suprageneric groups. Pollen morphology, seed coat structure, and the presence of petals and laticifers are especially important arbiters of relationships.

4.8.2. Phylogenetic relationships in *Phyllanthus* and relatives (Phyllanthaceae): Evidence from nuclear ITS and plastid *matK* sequences

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Phyllanthus, the largest genus of Phyllanthaceae, is a taxonomically challenging group due to the high species number (c. 850; including many pantropical weeds) paired with minute unisexual flowers. Webster's current classification is mainly based on architecture, pollen and floral morphology. Our previous molecular studies have shown *Phyllanthus* to be paraphyletic, with *Breynia*, *Glochidion*, *Reverchonina*, and *Sauropus* (totalling a further c. 450 species) embedded. This more detailed study analyses ITS and *matK* sequence data of over 100 species, including all subgenera except *Cyclanthera* and several undescribed and hitherto unplaced groups. The resulting trees are well resolved and reveal more complex relationships than previously thought. The unique architecture ('phyllanthoid branching') of most *Phyllanthus* taxa has been lost (and/or gained) several times independently. Subgenera *Isocladus*, *Kirganelia* and *Phyllanthus* are polyphyletic. Taxonomic divisions based on similar pollen morphology are confirmed, and related taxa share similar geographic distributions.

4.8.3. Systematics and biogeography of *Croton* and tribe Crotonaeae

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Croton is the second largest genus in Euphorbiaceae and was judged by Govaerts et al. (2000) to include 1,223 valid species. Our studies have focused on using nuclear and chloroplast DNA sequence evidence to evaluate the monophyly of the group and determine relationships within the genus and within tribe Crotonaeae. Based on the analysis of over 300 taxa sequenced for ITS and trnL-F, we hypothesize that the Crotonaeae consists of approximately six genera, with *Croton* remaining monophyletic with the exception of one small group, as follows: outgroup *Jatropha* (*Sagotia* + *Sandwithia*)+(Astraea + *Acidocroton*) + (*Brasilicroton* + *Croton*). Within *Croton* there is a basal trichotomy with one group comprising *Moacroton*, *Cubacroton*, *C. alabamensis*, and sect. *Corylocroton*; the second with *C. megistocarpus*; and the third a very large clade consisting of two New World clades and one Old World clade of *Croton*. Biogeographically, Crotonaeae and *Croton* both appear to have New World origins, and *Croton* is so far showing a single Old World radiation.

4.8.4. Phylogenetic relationships of *Mallotus*, *Macaranga* and related genera (Euphorbiaceae): Taxonomic consequences and Afro-Asian dispersal patterns

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The paleotropical euphorb genera *Mallotus* and *Macaranga* together comprise more than 400 species. Although currently classified in separate subtribes, the morphology suggests a close relationship between them. Furthermore, previous phylogenetic analyses have placed *Macaranga* nested within *Mallotus*, implying a need for drastic nomenclatural changes. A few additional mono- or bitypic genera also closely resemble *Mallotus* and should perhaps be merged with it. Plastid (*trnL* intron and *trnLF* intergenic spacer) and nuclear (nucpGS, ITS etc.) regions were sequenced to clarify the phylogenetic relationships among these genera. A combined analysis resulted in three strongly supported main clades: 1) a basal clade comprising *Cordemoya*, Madagascan *Mallotus* species and two Asian *Mallotus* sections; 2) a *Mallotus* s.s. clade including all other *Mallotus* species plus *Trewia* and *Neotrewia*; and 3) a *Macaranga* clade. Thus, *Mallotus* and *Macaranga* can be maintained as distinct genera, if the former is re-circumscribed. The phylogeny also indicates a dispersal from Asia to Africa and Madagascar in both the *Mallotus* s.s. and the *Macaranga* clades.

4.8.5. Phylogeny and biogeography of *Acalypha* (Euphorbiaceae)

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Acalypha, with about 450 species, is the fourth largest genus in Euphorbiaceae s.l. Its monophyly is strongly supported by a series of reproductive morphology characters, including small brevicolporate pollen, elongate vermiform anthers, no floral disc, and lacinate styles, all of which appear to be associated with anemophily. The genus is pantropical, with a few temperate species. We sequenced ITS and *ndhF* from over 100 species to reconstruct phylogenetic relationships within the genus. DNA sequence data support the monophyly of the genus and of subgenera *Acalypha* and *Linostachys*. Most other previously recognized infrageneric taxa are not monophyletic, however, and the characters used to define them are highly homoplastic. We have yet to find morphological characters that delimit most clades supported by the DNA sequence data. The phylogenetic results suggest that although the greatest species diversity is found in the neotropics, *Acalypha* originated in Africa where the greatest morphological diversity is found, colonized Asia several times, and colonized the Americas only three times.

4.8.6. Genetic diversity and evolution in *Manihot* (Euphorbiaceae)

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Cassava (*Manihot esculenta*, Euphorbiaceae), is the 6th most important crop globally and serves as the as main calorie source for over 500 million of the world's poorest people. Enhancement of cassava as a food crop must rely on the genetic resources from its closest wild relatives. The evolutionary relationships between cassava, its putative wild ancestor (*M. e. flabellifolia*) and a closely related congener (*M. pruinosa*) were determined by assessing DNA sequence variation at 3 nuclear genes and 5 SSRs. Populations of the wild taxa were collected throughout their range in Brazil. Genetic variation in cassava was assessed in a representative collection of cultivars obtained from CIAT. Both the SSRs and sequence data indicate that cassava is derived from *M. e. flabellifolia*. Genetic variation in cassava is a subset (<20%) of that detected in *M. e. flabellifolia*. These data refute the longstanding hypothesis that cassava is a "compilosppecies" derived from many hybridizing progenitor species. The geographic distribution of genetic variation in *M. e. flabellifolia* suggests that cassava was likely domesticated from southern Amazonian populations of this taxon.

4.9.1. A brief history of gymnosperms: diversity trends

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A comprehensive overview of gymnosperm families globally, extant and fossil, provides a new and distinctive perspective on the biodiversity trends of the group at higher taxonomic levels through their 375 million-year history. Total diversity figures are: 84 families, 37 orders, 10 classes. The 71 extinct families are based on reference whole-plant genera (with the focus on ovulate fruit), an approach dictated by the highly varying availability and grade of data on affiliated organs, and the relative rarity of microsporangiate fruit. The stratigraphic ranges of the 84 families of gymnosperm are plotted according to first and last appearance at the resolution of the geological stage. The biodiversity histogram based on these data clearly reveals four broad phases in the history of the gymnosperms: three periods of radiation and extinction, followed by an interval of stasis. The 'Secondary radiation' through the first 25 million years of the Triassic is clearly the most explosive and leads to the diversity heyday of the gymnosperms in the Carnian, with 30 families in 23 orders and 8 classes.

4.9.2. The Gnetales: fossils and phylogenies

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The Gnetales have often been considered a small remnant of a former greater diversity, but few megafossils have been found. We

have described Early Cretaceous fossils from Portugal, USA and China which share several unique features with modern *Ephedra*, such as the peculiar naked male gametophyte and papillae on the seed envelope. The paucity of information in most gene regions has resulted in a young age (8-32 Myr) for modern species in molecular dating analyses. However, the fossils are often more or less identical to modern *Ephedra* in vegetative morphology and detailed reproductive structures, and indicate that the crown group could be much older (110-127 Myr). While the relationship of *Ephedra* as sister to *Welwitschia* plus *Gnetum* is clear, the relationship of Gnetales to other seed plants is still not fully understood. We found conflicts within two chloroplast genes, and different topologies can be obtained from different substitution models and character codings. Taxon sampling error may be another problem. Seemingly well-supported, but conflicting, topologies can be obtained simply by substituting a few terminals in bayesian and parsimony analyses of *rbcl*.

4.9.3. Reassessing seed ferns in seed plant systematics, evolution and phylogeny

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Seed plants first evolved in the late Devonian and include a large number of extinct groups as well as five extant lineages (cycads, ginkgos, conifers, Gnetales, and angiosperms). This presentation makes a holistic assessment of seed plant relationships, evolution and phylogeny by presenting results of a new morphological cladistic analysis of lignophytes (seed plants plus their progymnosperm ancestors). The analysis includes a significantly expanded taxon selection, augmented by reassessments of the individual anatomical, morphological and developmental characters used. Results show the importance of seed ferns in determining the higher level relationships within seed plants, and emphasise the importance of seed ferns for the origins of extant seed plant lineages. Results place the extinct seed plant *Caytonia* as sister to angiosperms, and shows conifer to be monophyletic and to exclude Gnetales. Taxon selection is demonstrated to have a profound effect on the topologies generated by the analysis, and highlights the need for further whole plant reconstructions from fossil data to fill known gaps in fossil record of seed plants.

4.9.4. Systematics of the most ancient conifers

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Conifers play a central role in seed plant phylogeny but phylogenetic relationships of living and fossil conifers are unresolved. Recent studies have cast serious doubts on the hope that deep branches within seed plants, can be resolved using only living species. The fossil record of the most ancient conifers (Upper Paleozoic) is most often characterized by fragmentary branching systems, that do not allow us (1) to assign newly described specimens to a species with confidence, or (2) to score enough characters to conduct phylogenetic analyses. New whole plant reconstructions from the Hamilton Quarry, Kansas have been used in cladistic analyses of morphological characters, and these support some of the previously proposed relationships among the most primitive conifers and other Paleozoic coniferophytes. However, they question the validity of all previously proposed families for walchian conifers. This whole plant approach suggests the need for a complete systematic reevaluation of the most ancient conifers and their relationships to conifer families with living species.

4.9.5. A phylogeny of Cycads (Cycadales) inferred from chloroplast *matK* gene, *trnK* intron, and nuclear rDNA ITS region

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Phylogenetic relationships among the 3 families and 12 living genera of cycads were reconstructed using three markers: the chloroplast *matK* gene and *trnK* intron and the nuclear *ITS/5.8S rDNA* sequence. All datasets indicate that Cycadaceae is remotely related to other cycads, in which *Dioon* was resolved as the basal-most clade, followed by *Bowenia* and a clade containing the

remaining 9 genera. *Encephalartos* and *Lepidozamia* are closer to each other than to *Macrozamia*. The African genus *Stangeria* is embedded within the New World subfamily Zamioideae. Therefore, *Bowenia* is an unlikely sister to *Stangeria*, contrary to the view that they form the Stangeriaceae. The generic status of *Dyerocycas* and *Chigua* is unsupported as they are paraphyletic with *Cycas* and the *Zamia*, respectively. Nonsense mutations in the *matK* gene and indels in the other two datasets lend evidence to reinforce the above conclusions. According to the phylogenies, past geography of the genera of cycads and the evolution of character states are hypothesized and discussed. Re-delimitations of Zamiaceae, Stangeriaceae, subfamily Encephalartoideae and subtribe Macrozamiineae are necessary.

4.9.6. Evolution of female cones of extant conifers - Conflicting data from morphological and molecular studies?

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In spite numerous cladistic analyses that try to resolve the phylogeny of conifers and to find the origin of this group, a convincing model of this group is still lacking. Moreover recent molecular studies based on several genes, support topologies, which seem to be in conflict with morphological analyses. Crucial for the reconstruction of conifers phylogeny is the interpretation of the ovuliferous dwarf-shoot of the female cones. Although it is generally accepted that the female cones of the conifers represent compound structures, a convincing model of the evolution of the different cones in the extant groups is still lacking. Based on our investigation on the female cones of extant conifers, we discuss the critical boundary of „homology“ in respect to the female cones of conifers. We believe that it is fundamentally for reliable cladistic analyses to define frame homologies and detail homologies to avoid topologies that are based on the summation of analogisms. We present alternative scenarios for the evolution of the female cones of the conifers and illustrate that the woody cone was evolved at least twice.

4.9.7. Molecular control of seed cone development in Norway spruce (*Picea abies*)

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We have shown that MADS-box C-type genes are expressed in the ovuliferous scale of the female cone, and together with B-type genes in the microsporophylls of the male cone. In the ABC-model of flower development C-gene expression results in pistil and C together with B expression in stamen development. Hence, our data indicates that, despite great morphological and evolutionary divergence between the cone and the flower, common genetic pathways regulate reproductive organ development in angiosperms and gymnosperms. We have now performed a detailed analysis of the expression patterns of a set of MADS-box and LFY genes during the development of the female cone. Our current study corroborates previous data, but adds also putatively conifer/gymnosperm specific factors without apparent orthologues in angiosperm genomes, that may act together or parallel with the B and C-genes in cone development. Based on these expression profiles we present a model on how MADS- and LFY transcription factors may act to regulate spruce cone development.

4.10.1. The invasion of *Bromus tectorum* in North America: the role of pre-immigration evolution

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The annual grass *Bromus tectorum* L. (cheatgrass or downy brome) is one of the most widespread and destructive plant invaders in North America. As an almost exclusively cleistogamous species, the opportunity for post-immigration out-crossing and selection among new genotypes has probably been minimal. Instead this grass's continental spread and proliferation illustrates amply that invasions usually arise from multiple introduction events, each with a mixture of genotypes that are the product of random genetic sampling in different parts of the donor range(s). If out-crossing is minimal or nil, any subsequent invasion occurs through pre-adaptation. Although at least 7 genotypes (introductions) can be detected, only three are widespread. The on-going invasion in the mid-continent largely consists of the most common genotype detected in Eurasia. In striking contrast, the

most destructive invasion in the arid Intermountain West has been caused by a genotype (Got-4c) that is rare or undetected east of the Rocky Mountains. The evolution that explains this grass's invasion across North America occurred before the first immigrants arrived.

4.10.2. Evaluating the role of different evolutionary forces during a biological invasion: Is natural selection enough?

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One explanation for invasiveness is that selection resulting from enemy escape favors a shift in investment from defense to traits enhancing growth and reproduction. *Silene latifolia* was introduced from Europe (EU) to North America (NA) approximately 200 years ago. Field studies and common garden experiments conducted in EU and NA have revealed major differences between the two continents: 1) *Silene* has escaped a number of specialist enemies during colonization and the resulting damage levels are lower in NA; 2) NA plants display a 'weedier' and more reproductively prolific phenotype, and; 3) NA plants invest less in defense and have lower resistance to fungal infection and fruit predation. These results are consistent with the notion that the invasive NA phenotype has evolved at the expense of defensive abilities due to altered selection pressures in enemy-free NA. Yet, while it may be parsimonious to invoke natural selection as the driving force, the presence of genetic structure in EU and certain aspects of *Silene*'s colonization history suggest it is also important to consider the role of genetic drift in the evolution of novel phenotypes during invasion.

4.10.3. Preadaptation or adaptation - which is more important in plant invasions?

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Of the many plants introduced by humans into new areas, why do a few become abundant? Although there have been many attempts to find an ecological answer to this problem, no plant traits have been identified that are consistently associated with an ability to invade; indeed, many biologists now argue that the capacity of a species to adapt may be more important than any particular ecological characteristic. In this presentation, we evaluate the present state of the adaptation versus preadaptation debate. We argue that one reason for the present confusion is a failure to treat invasions as dynamic processes in which selection pressures change over time. While the initial success of a species may be due to ecological traits associated with the introduced genotypes, adaptation to changing conditions is likely to be important if the species is to expand its range and establish itself in a wider range of communities.

4.10.4. The evolutionary consequences of plant invasions

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Invasive species provide ideal systems to understand evolutionary processes that determine and accompany expansion of a species. Invasions have two fundamental evolutionary consequences; they can result in rapid evolution of introduced populations, and they can promote reticulate gene flow with related taxa at the population, ecotype, or species level. It is clear that invasions can result in rapid evolution via a number of genetic mechanisms. Examples are provided for the geographic and evolutionary origin of invasive species, identification of parental genotypes involved in the formation of new invasive species, possible genetic exchanges between invasive and native species, genome evolution of invasive species compared to related non-invasive species, the molecular basis of adaptive traits that may promote invasiveness, and the importance of neutral vs. adaptive processes that have accompanied genome evolution of invasive species. The genetics of invasive species are interesting and important from an evolutionary perspective, provide an ideal context for the synthesis of ecology and genetics, and merit consideration at the management level.

4.10.5. Genetic differentiation versus morphological plasticity in *Verbascum thapsus* invading across an elevation gradient

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Since its introduction from Europe to Hawaii nearly a century ago, the biennial weed *Verbascum thapsus* (common mullein) has invaded across a broad elevation gradient, exhibiting substantial morphological and life history variation, including an unusually high frequency of fasciation. To assess the contributions of genetic differentiation and phenotypic plasticity to the observed phenotypic variation, we established field common gardens in Hawaii and Europe using seeds from seven populations collected across an elevational range of 1000 m in Hawaii. Initially, there was significant variation among populations and within populations for germination rate, rosette diameter and number of leaves. However, beyond the end of the first growing season, there were no significant differences in plant size or other morphological measures among populations, and the fasciated morphology was not heritable. By the third year, most plants surviving in Hawaii remained in the rosette stage, indicating a delayed life history. Observed morphological differences across the elevation gradient appear to be mainly due to plasticity in response to environmental conditions.

4.10.6. Evolution of life cycle in invasive plants and implications for biocontrol

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Rapid adaptive evolution of invasive plants during the process of range expansion might also affect plant-antagonist interactions. We will specifically explore how altered selection in the new range might influence the evolution of life cycle and how this might affect subsequent biological control efficacy. Using the model species *Centaurea maculosa*, introduced from Central Europe into North America during the late 19th century, we will explore the hypothesis that a polycarpic habit (flower several times) may have been negatively selected by specialist herbivores in the native range, resulting in predominantly monocarpic populations (plants die after flowering), but positively selected in the introduced range where the specialist herbivores are absent. Invasive populations should, thus, be highly susceptible to introduced biocontrol herbivores and their release could reverse the presumed evolutionary shift towards a polycarpic life cycle in the introduced range. We will discuss available evidence of this hypothesis and present recently initiated collaborative studies linking biological control and evolutionary biology.

4.10.7. Interspecific hybridization between invasive and native plant species

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It has been argued that hybridization, either between formerly isolated populations of the same species, or between invasive and native plant species, may provide a source of genetic variation leading to the evolution of increased invasiveness. During my talk I will focus on interspecific hybridization between invasive and native species in Central Europe. A quantitative estimate of how many invasive species are actually known to hybridize and the results of a case study in *Rorippa austriaca* (Brassicaceae) will be presented. *R. austriaca* is native in southeastern Europe and western Asia and invasive in Central Europe. Hybridization between *R. austriaca* and the closely related *R. sylvestris* has been analysed using molecular markers (chloroplast DNA, AFLP). This system provides the rare opportunity to analyse the effect of age on hybrid zone structure since *R. sylvestris* is native in large parts of Eurasia. In a comparative analysis of genetic variation in five different hybrid zones, older hybrid zones in the native range of *R. austriaca* showed higher degrees of admixture than young hybrid zones in the invaded range.

4.11.1. The role of plant diversity for grassland ecosystem dynamics under global change

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Grasslands are a major contributor to the biosphere - atmosphere exchange of radiatively active trace gases in Europe, with fluxes intimately linked to management practices. Plant-plant (e.g. competition and facilitation), plant-soil and plant-herbivore interactions contribute to the regulation of C and N fluxes in grassland ecosystems and mediate community and ecosystem responses to external drivers like climate and land use changes. We discuss results from long term experiments and from mesocosms, exposed or not to elevated CO₂, which show the role of above and below-ground plant functional traits for C and N cycling in semi-natural grasslands. We conclude that mechanistic models coupling community dynamics, food webs and biogeochemistry will be required to improve our understanding of global change impacts on semi-natural grasslands. Some first simulation results obtained with such a model (GEMINI) are discussed.

4.11.2. Changes in plant community structure of European grasslands in response to elevated CO₂ and management

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We have tested the hypothesis that long-term exposure of mixed species communities of grassland to elevated CO₂ will result in significant changes in structure. We report on a series of experiments at six locations in Europe where we have exposed local grassland communities to elevated CO₂ in combination with management regimes of either different cutting intervals or different fertiliser inputs. Monoliths of semi-natural grasslands were exposed to elevated CO₂ for at least two whole growing seasons using mini-FACE exposure systems. We show for all the grassland communities that increased atmospheric CO₂ concentration leads to species-specific responses, which alter the plant community structure in different ways, depending upon the management provided. We conclude that the dominance and successional patterns of mixed species grasslands will be subject to gradual changes as the atmospheric CO₂ concentration rises. Also, the management applied to multi-species grasslands may need to be revised in order to maintain (or increase) herbage quality and plant diversity.

4.11.3. Identification of spatial distributed carbon sink within a grassland canopy

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Assimilatory and respiratory activity of grassland community shows distinctive spatial and temporal heterogeneity. The reason for this can be found in the different ontogeny stage of participated grass species and, more important, the spatial differentiation of plant canopy, which is responsible for different solar radiation regime within canopy. Thus, final carbon assimilatory activity of whole grassland canopy is possible sense as clusters of active or inactive assimilatory points. The basic investigation on carbon deposition within grassland canopy is based on comprehensive investigation of photosynthetic characteristic (based on gasometry and chlorophyll fluorescence measurements) of individual grass species within grassland stand. It makes possible to evaluate basic response function of assimilation to absorbed quantum yields and to temperature, respectively. The whole stand assimilatory/respiratory balance, i.e. Net Ecosystem Exchange of investigated stand, is possible to evaluate by the application of eddy covariance technique. To be able to evaluate spatial distribution of individual components of NEE the combination with remote sensing and appropriate parameters (PRI for example) is of great advance. An example of this type of spatial investigation of carbon sinks within a mountain grassland canopy is presented.

4.11.4. Net ecosystem dynamics of grasslands on different soils in Hungary

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Net ecosystem exchanges at two grassland sites have been measured by the eddy covariance technique in 2003 and 2004. At the Bugac site the vegetation is a temperate semiarid sandy grassland. At the Mátra site the vegetation is seminatural grassland on a clay soil. Management at the Bugac site is based on grazing, while in the Mátra mountains the management is partly dependent on the weather, mainly on rainfall. The two grasslands proved to be different in their drought tolerance in terms of NEE in the years of 2003 and 2004. Vegetation at the sandy grassland site started to grow earlier and showed better drought tolerance than the mountain grassland. One of the factors causing these differences is that the sandy grassland lies more to the South, so the vegetation period starts earlier, resulting in a longer growth period with optimal water supply. On the other hand the heavy soil at the mountain site, dominated by the clay fraction is characterized by worse water economy than the sandy soil. The sandy grassland proved to be a stronger sink in the wetter year (2004) than the mountain grassland, and tolerated the 2003 extreme drought also better than the mountain grassland.

4.11.5. Mediterranean grasslands under elevated carbon dioxide

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The response of natural Mediterranean grasslands to elevated atmospheric carbon dioxide concentrations was studied in different experiments done in the open, using different fumigation techniques. The effect of long term carbon dioxide enrichment on the grass species growing in a carbon dioxide spring was studied both in situ, as well as transplanting turves of the grassland community in miniFACE fumigation rings. The effect of fumigation was scarce, while a stronger effect was exerted by fertilization. Scarce effects of elevated carbon dioxide on above ground productivity were also evidenced in a grassland fumigated in situ by miniFACE rings. In this experiment, the variations of ground cover of perennial and annual species, and the changes in their phenological cycles, were also recorded. Perennial species showed some increase in ground cover, while annual species displayed a quicker phenological cycle. Yet the response was typically species-specific. Most of the examined species showed a reduction in transpiration.

4.11.6. From Mediterranean to desert ecosystems: an integrative approach for climate change research

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We established a novel and integrative project focused on the main limiting factor driving the ecosystem functioning in desert and Mediterranean ecosystems: changes in precipitation patterns. Our multidisciplinary project employs, in addition to a natural aridity gradient, climate manipulations that intend to close the gap between descriptive and empirical research approaches, and theoretical modeling. The goals of the project are: (1) to provide empirical data for modeling and predicting ecosystem responses to climate change in an environmentally sensitive area, and (2) to obtain knowledge of the factors that control ecosystem functioning and structure in the face of global climate change. Our contribution is based on an integrative study of soil, overland-flow, vegetation and landscape processes affecting the ecosystem, combined with socio-economic methodology. Thus, a holistic approach is applied, where we assess the impact of climate changes on plant and human communities.

4.11.7. Effects of land use change on temperate seminatural grasslands

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The specific objectives of this study were to determine the effects of different land use changes (extensive grazing, fertilization and

irrigation) on the botanical and soil composition, production and greenhouse gas exchanges and to provide data for model development. Experimental work has been carried out for 3 years in two characteristic semi-natural temperate grasslands of Hungary. The selected loess steppe located in the north-central hilly part of the country (near Isaszeg) from where monoliths were transplanted to Gödöllő, while the sand grassland situated in the Hungarian Plain (around Bugac). Small grassland plots were positioned along two transects at fertilized and irrigated sites. At the Isaszeg site, mineral fertiliser was applied once a year at the beginning of the vegetation period, while top spray irrigation was operated continuously during each vegetation period at the Gödöllő site. On the plain site an area of 6 ha was fenced off from the extensively grazing cattle herd. The observed changes were strongly correlated to climatic conditions, emphasized the importance of the water regime.

4.12.1. Living inside rock - survival strategies of the lichen *Verrucaria rubrocincta* in a desert habitat

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The lichen *Verrucaria rubrocincta* occurs in one of the most extreme habitats of the Sonoran Desert, inside caliche plates on desert pavements. These surfaces are exposed to high solar radiation. In the summer thalli survive air temperatures regularly exceeding 40 °C. Rock surface temperatures are even more extreme; reaching 60 °C during the day, rarely falling below 30 °C at night. In the absence of precipitation, relative humidity attains a maximum c. 30 minutes after sunrise, then dropping rapidly. On average 5 to 7 days per month have rainfall; relative humidity exceeds 75% only during winter months. The endolithic anatomy can be interpreted as strategy against these extremes. The lichen deteriorates its substrate establishing entirely inside the rock simultaneously inducing biomineralization of a calcite surface. This new formation is a modification of the pseudocortex previously described from endolithic lichens. It occurs in close proximity to substrate deterioration caused by hyphae forming a pseudomedulla. Using x-ray diffraction and isotope analysis, we propose a simple model as first step towards understanding the interaction of both processes.

4.12.2. Symbiont selection in lichens with different modes of propagation

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Trebouxoid algae are the most common photobionts in the large order Lecanorales. Our overall phylogeny of these photobionts and an increased sampling of lichen species provide new insights in patterns of symbiont selectivity. These data show that there are significant differences in the photobiont selectivity in different lichen species. Here we present our growing data about symbiont selectivity in *Physconia*, where we focus on the common species *Physconia distorta* and *Physconia enteroxantha*. The two species differ by their propagation mode: *Physconia distorta* is mainly fertile with ascospore dispersal, while *Physconia enteroxantha* is sterile and jointly distributes both symbionts. These species are restricted to symbiosis with *Trebouxia impressa*. We observe a higher variation of *Trebouxia impressa* in Mediterranean habitats. Haplotype networks will be presented for *Trebouxia impressa* and different photobionts in other lichens. These show the extremely wide geographic distribution of certain common algal genotypes, while others seem to be rarer or limited to certain environmental conditions.

4.12.3. Colonization strategies and symbiont selectivity of lichens on leaves

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Finding the appropriate partner is the initial step in the life cycle of exosymbiotic associations. Thus the establishment of symbioses depends on the presence of compatible partners in more or less close vicinity of germinating propagules. The optimization of interactions between the partners and the efficiency to acquire a

partner are major forces towards the evolution of symbiont selectivity patterns and diverse life history strategies. In principle, symbionts may either passively wait or actively move towards their potential partners, which are spread in the habitat. In the lichen symbiosis, algae usually lack active dispersal, whereas the fungal partners capture photobionts by diverse ways. Examples for different propagation and colonization strategies and the consequences on symbiont selectivity are discussed with examples. This will focus on lichen associations on leaves, which are an outstanding model due to their short life cycles.

4.12.4. Where does the lichen photobiont come from? II. Photobionts of the *Acarosporium sinopicae*.

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Lichen fungi reproducing by spores, need to find compatible algae in order to form a new lichen thallus (re-lichenisation). Sexually reproducing lichens can only grow, where suitable photobionts are available and thus the occurring photobionts determine to some extent the composition of lichen communities. A possible source of photobionts may be free-living populations of algae (the existence of these is controversial) or parts of thalli of other lichens (e. g. vegetative diaspores, thallus fragments) which contain the compatible photobiont. To investigate possible sources of photobionts, it is necessary to know the algal partners of all lichen species occurring in the same community, because the taking over of photobionts is only meaningful, when the lichen fungi in question form lichens with the same algal species. These relations may vary between different communities and therefore, it is important to know the composition of the photobiont pool in the lichen communities. Such a photobiont composition will be discussed using light microscopical and molecular data (ITS rDNA sequencing).

4.12.5. Can culture experiments with lichen fungi and algae/cyanobacteria elucidate essential morphogenetic steps in lichen life cycles?

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Experiments to separate and recombine lichen symbionts can be considered as the "birth" of "experimental lichenology". As most early manipulations with lichen bionts did not have success to reconstitute a fully developed thallus from its bionts (resynthesis) or to grow a lichen thallus in culture, experimental lichenology remained a nearly unexplored field for decades. With new concepts and novel methodological approaches we are able to do "case" studies in our laboratory and simulate major steps in the establishment of the lichen symbiosis. Three types of investigations will be reported: 1) How can we handle a three partite symbiosis and complex thallus morphologies under lab conditions? 2) What are the differences between the different starting "units" for re-establishing a lichen thallus by spores and algae (sexual reproduction) and by diaspores (vegetative propagation)? 3) Do aposymbiotically grown mycobionts form fruiting bodies? Is reproduction in culture induced by external factors? The presentation will give an overview both about the most successful and also the "failure" trials to reconstitute lichen life cycles under laboratory conditions.

4.12.6. Acclimation of the photobiont and mycobiont partners in the foliose lichen *Xanthoria parietina* to seasonal changes in solar radiation

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Xanthoria parietina (L.) Th. Fr. is a sunshine tolerant foliose lichen. We studied the capacity of both the green-algal photobiont *Trebouxia* and the mycobiont to acclimate to seasonal changes in sunlight levels. We firstly focused on the fungal partner, specifically, on the seasonal acclimation of the sun-screening orange cortical anthraquinone, parietin in a natural population. A distinct seasonal variation, with low parietin contents at winter solstice increasing two fold at summer solstice was recorded. The annual cycle is consistent with a solar radiation protective function of parietin operating also on a temporal scale. The second part studied the seasonal dynamics of various chlorophyll fluorescence parameters and xanthophyll-cycle pigments as a possible indicator of photobiont acclimation to solar radiation. The seasonal course in

susceptibility to photoinhibition was also evaluated. Both parietin-free and -intact thalli were measured. Results showed that the photobiont also acclimates to seasonal solar radiation levels. Parietin-free thalli were more seasonally susceptible to photoinhibition than controls.

4.13.1. The physiological functions of nitrogenous osmolytes accumulated by higher plants subjected to osmotic stress

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The yield lost occurring in crops submitted to drought and salinity often associated with high temperature is mainly related to the damages induced at the cell level by dehydration. Upon exposure to these environmental stresses higher plants are known to accumulate low molecular weight organic compounds. For a long time, these compounds have been supposed to behave as osmolytes contributing to the re-establishment of the turgor needed for survival and hopefully for a low growth rate. Considerable efforts have been directed over the past four decades to investigate, *via* correlative physiology and genetic engineering the contribution of osmolytes (i.e. the so-called compatible solutes) to osmotolerance of higher plants. However their physiological function(s) still remain(s) a matter of debate. This oral presentation will be devoted to the changes induced under stress conditions in specific metabolic pathways which allow accumulation of nitrogenous substances expected to participate in osmoprotection. These pathways will be evaluated in terms of cost and benefits for plant cells. In addition the relevance of the criteria used to assess the compatibility of their terminal products will be discussed. Recent findings concerning the toxicity of proline and glycine betaine, when supplied to non-stressed plants will be shown to stress that the dogmatic compatibility of these solutes remains debatable. This should be of special interest because a number of crop plants are currently being genetically modified at the level of relevant genes involved in osmolytes synthesis with the aim to improved tolerance to osmotic stress. Data suggesting that the so-called compatible solutes do not behave just as inert osmo-balancers but also as signal molecules that counteract injuries induced by osmotic stress will also be presented.

4.13.2. Hydraulic and hormonal signals are involved in plant adaptation to water stress

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Many stress factors affect only certain parts of plants but cell metabolism may change very rapidly in cells located far from the place of stress factor action. *Mesembryanthemum crystallinum* L., a facultative halophyte, shifts from C3-type of photosynthesis to *Crassulacean* acid metabolism (CAM) under soil drought or salinity. PEPC gene expression in leaves was induced in 4-6 hours after the decrease of water potential in rooting medium with NaCl or PEG 6000 which witnessed for CAM initiation. But at this time mass water flow from roots to aerial parts of plants was strongly limited due to reversal water potential gradient. Nevertheless, as early as in 10 min of stressor action, the sharp decrease of turgor pressure in leaf cells was registered while ABA started to accumulate there not early than in 6-9 hours. It has been concluded that hydraulic signal serves as a primary signal of inter-organ communication being followed by much slower chemical signal (ABA) under acute water stress.

4.13.3. Lipid signalling pathways differently modulates proline accumulation in *Arabidopsis thaliana* and in *Thellungiella halophila*

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Accumulation of proline is a wide spread plant responses upon environmental stresses, especially drought and salinity. Despite the importance of proline accumulation in plant adaptive responses to stress and extensive knowledge on proline metabolic pathways, signalling cascades involved in the regulation of these pathways are still unclear. Our current efforts are focused on elucidating the

signalling pathways involved in the regulation of proline metabolism, and understanding the role of proline accumulation in whole-plant response to osmotic-stress tolerance in the two model species, the glycophyte, *Arabidopsis thaliana*, and the halophyte, *Thellungiella halophila*. We provide experimental evidence that positive and negative regulators are involved in the fine regulation of proline metabolism under hyperosmotic stresses. Different *Arabidopsis* mutants tagged in genes related to these signalling components are under molecular and phenotypic analysis. Our data suggests the implication of lipid signalling in the regulation of proline metabolism in *A. thaliana* and *T. halophila* but in an opposite fashion.

4.13.4. A role of the plastid compartment in age- and methyl jasmonate-induced senescence

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Senescence occurs naturally as last step of leaf development (age-induced senescence). However, it can also be induced by certain phytohormones, such as abscisic acid, brassinolides and jasmonates. Here we studied ultrastructural changes occurring in the plastid compartment in conjunction with changes in expression of plastid and nuclear genes encoding photosynthetic proteins during natural and jasmonate-induced leaf senescence in barley. Both senescence types were characterized by a dramatic decrease in the number and size of chloroplasts per cell and a reduction in expression of photosynthetic proteins. Thylakoid disintegration was accompanied by the formation of plastoglobuli. Our results unveil a hitherto unrecognized role of the plastid envelope in programmed organelle destruction during either senescence type and identify the envelope membranes as a source of jasmonate precursors and origin of novel signal transduction pathways.

4.13.5. Salt tolerance in plants: the ASR1 case

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Exposure of plants to salinity results in a massive change in gene expression. The role of many of these gene products is not known. It is therefore necessary to study the role of stress-induced proteins to better understand plant stress response. Tomato ASR1 is a plant specific protein, whose role could not be deduced from sequence homology. The levels of *Asr1* transcripts and ASR1 protein are transiently increased in response to salt-stress, osmotic-stress or treatment with abscisic acid. ASR1 is localized in both cytoplasm and nuclei. Purified ASR1 protein possesses a sequence specific zinc-dependent DNA-binding activity. The novel DNA and zinc binding sites of ASR1, and its protein and oligomeric structure were studied. Tobacco and *Arabidopsis* plants over expressing ASR1 demonstrated improved salt tolerance: they had improved germination and improved survival in the presence of salt than non-transformed plants. Overexpression of ASR1 resulted in the induction of other genes under control conditions. We suggest that ASR1 protein play a role in the signal transduction pathway resulting in the alternation of gene expression upon salt-stress.

4.13.6. Adaptive cellular autophagy (ACA) as new marker of plant stress tolerance and plant diversity

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Most abiotic stresses induce water deficit in the cells. As a response, plants escape water loss. However, over long periods of water shortage, adaptation occurs at the cell level and allows plants to tolerate drought-stress. We have shown that water stress-induced cell membrane damage was correlated with the stimulation of hydrolytic activities (lipolytic and proteolytic). To study drought-induced protein catabolism, we build an experimental system responding to dosage effect that allowed us to relate our results with field drought-tolerance capacities. It includes related species of the *Fabaceae* family (*Phaseolidae* tribe) with various degrees of drought tolerance. In our experiments, cultivars are submitted to four level of controlled water stress and followed by rehydration. Results showed that water stress induced a rise in proteolytic and lipolytic activities paralleled with an increase in the expression of the

corresponding genes. Tolerant plants are able to express protective genes, such as kininogen-like cystatins involved in maintenance of adequate cell catabolism to anabolism balance. On the contrary susceptible plants showed higher hydrolytic activities and lower inhibitor transcript contents leading to shortening of cell life and plant death.

4.13.7. Improvement of crop tolerance to drought and salinity: Application of biotechnology and nuclear-related techniques

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The joint FAO/IAEA division is evaluating opportunities for using nuclear and related technologies to improve crop tolerance to abiotic stresses such as drought and salinity. An important project is currently being implemented for the identification and development of crop germplasm with superior resource use efficiency and nutritional value and adapted to harsh environments. Two coordinated research programs have been recently launched under this project dealing respectively with (i) the identification and pyramiding of mutated genes, and novel approaches for improving crop tolerance to salinity and drought, and (ii) the selection for greater agronomic water use efficiency in wheat and rice using carbon isotope discrimination under drought and saline environments. This paper reviews the recent progress made at the joint FAO/IAEA division in deciphering the complexity of drought and salinity problems and developing drought- and salt-tolerant varieties and presents future perspectives for the use of isotopes and nuclear techniques for the improvement of crop adaptation to these stresses.

4.14.1. A counterrevolution in historical biogeography: the resurrection of oceanic dispersal

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Geographical distributions of terrestrial or freshwater taxa that are broken up by oceans can be explained by either oceanic dispersal or vicariance. The validation of plate tectonics theory provided a global vicariance mechanism and, along with cladistic arguments for the primacy of vicariance, helped create a view of oceanic dispersal as an explanation of last resort. I review recent work that suggests that the importance of oceanic dispersal has been strongly underestimated. In particular, molecular dating of lineage divergences favors oceanic dispersal over tectonic vicariance as an explanation for disjunct distributions in a wide variety of taxa. Various other kinds of evidence also indicate unexpectedly high frequencies of oceanic dispersal. If vicariance biogeography was a revolution, we are now in the midst of a more strongly dispersalist counterrevolution. This new view implies that biotas are more dynamic and have more recent origins than had been thought. A high frequency of dispersal also suggests that a fundamental assumption of many studies -- that vicariance is a priori a more likely explanation than dispersal -- needs to be re-evaluated.

4.14.2. The influence of dispersal on neotropical biogeography

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Arrival of lineages by long distance dispersal has strongly influenced the composition of the neotropical flora. However, the role of dispersal in shaping distribution patterns within the neotropics is less clear. For example, even though explanations for the disjunct distribution of seasonally dry neotropical forests favor vicariance, the floras of Central and South America appear to be linked either by population migration or dispersal. Here, we review paleoecological data and phylogenetic studies on several neotropical plant groups. In particular, Bignoniaceae (Bignoniaceae), the largest clade of lianas in the neotropics, occurs in many ecological zones and provides an excellent model for investigating neotropical biogeography. We use a broad-scale phylogeny and species range modeling to investigate distribution patterns in this morphologically diverse clade. These data favor a complex picture, in which dispersal and population migration have been important factors for shaping distribution patterns. Understanding the relative contribution of these different processes will require detailed phylogeographic analyses of many different groups.

4.14.3. Seed dispersal and species distributions in tropical forests today and in the future

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Dispersal ability is expected to relate to both historical species distributions and current and future changes in these distributions in response to anthropogenic changes in habitat extent and spatial pattern. We quantify the relationship of dispersal mode and estimated dispersal distance to estimated range size among neotropical woody plant species. We then use 12 years of seed rain data within a mapped plot including primary and secondary forest to estimate and relate seed production, seed dispersal, and ability to disperse into cleared land and secondary forest for 25 Puerto Rican tree species. Further, we analyze seed rain and seedling data from protected and hunted forests in Panama to assess how hunting differentially influences dispersal of tree species varying in seed size and dispersal mode. Finally, we integrate these results with data on the relative frequencies of different dispersal characters among tropical woody plant species and with projections of the future area and distribution of intact and disturbed tropical forests to predict how seed dispersal characters and species distributions are likely to covary in the future.

4.14.4. Wind as a long-distance dispersal vehicle in the Southern Hemisphere

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Although Southern Hemisphere landmasses are separated by considerable oceanic barriers to biotic exchange, they share strong biotic affinities. A long-lasting debate resolves around whether these affinities are the result of past Gondwanan connections or recent long-distance dispersal events. We used global winds coverage from the NASA SeaWinds scatterometer to test whether biotic similarities of Southern Hemisphere landmasses conform better with (i) the anisotropic long-distance dispersal (LDD) hypothesis, which predicts that connection by "wind highways" increases biotic similarity, (ii) a direction-independent LDD hypothesis, which predicts that biotic similarity among sites increases with geographic proximity, or (iii) a vicariance hypothesis, which predicts that biotic similarity increases with decreasing separation time between landmasses. We find a stronger correlation between floristic and faunistic similarities and wind connectivity than with the other two hypotheses, a result that supports the idea of wind as a dispersal vehicle for many organisms in the Southern Hemisphere.

4.14.5. Interfacing paleobotanical with molecular/systematic data in biogeography

A. K. Graham;
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Biogeography is in a transition phase in that plate tectonics, climatic change, vicariance, and dispersal are acknowledged as important for understanding patterns of distribution, while more subtle refinements are emerging from a synthesis of paleobiological and molecular data, and from a systems approach that integrates observations from seemingly unrelated disciplines. The syntheses reveal, for example, that in *Rhizophora* different histories are suggested by the fossil and preliminary molecular evidence. Both are equally valid and probably represent the typical situation as both approaches are utilized. The systems approach reveals that some African Elements in the Caribbean-South American region may have originated from increased hurricane numbers and intensity during warm intervals of the past (e.g., Paleogene, Middle Pliocene), a relationship documented by modern global warming. Full integration of paleobotanical and modern systematic evidence now requires new efforts to database the global plant fossil record.

4.14.6. Constructing biogeography: patterns of dispersal in the Southern Hemisphere

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Molecular phylogenies and divergence time estimates indicate that long-distance dispersal has played an important role in shaping

southern hemisphere floras. Although molecular analyses have provided many important insights into the origins of floristic similarities between Australia, New Zealand, and South America, these studies may be limited by extinction. Specifically, extinction of populations established following dispersal will confound inferences about dispersal frequency and pathways. Although uncertainty about the historical biogeography of specific lineages is always likely to remain, simulation studies may offer an important tool for understanding biogeography. Here we use a neutral biogeographic model to simulate diversification and dispersal of a lineage within a spatial scenario that represents the southern hemisphere over the last 20 my; in particular we explore whether dispersal could result in a tree topology consistent with that expected from vicariance. Our results are consistent with observations from molecular and fossil data, but also suggest novel insights into dispersal in the southern hemisphere.

4.15.1. Basic and applied plant ecology research in tropical field stations in Mexico: perspectives, challenges and opportunities.

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The most urgent challenge in tropical forest research is exploring the interface between ecology and the socio-economic forces that cause ecosystem transformation and degradation. World-wide, deforestation and land use change causes the rapid loss of tropical forests with dramatic, negative consequences for biodiversity, ecosystem function, and environmental services. Biological reserves play a major role in protecting tropical forests, but alone do not ensure forest preservation. There is an urgent need to generate scientific knowledge and tropical forest management techniques that promote human well being in a context of ecological sustainability. The next step is the adoption of an interdisciplinary research approach, where the natural and social sciences join in an effort to provide the basis for sustainable forest management. Studies being conducted at Los Tuxtlas, Chamela and Chajul Field Stations in Mexico will be used to show how interdisciplinary research teams can develop scientific basis for sustainable tropical forest management.

4.15.2. Tropical forests of Asia: long-term changes in dynamics

S. J. Davies;

Center for Tropical Forest Science-Arnold Arboretum Asia Program, Cambridge, MA, United States.

Human impacts on biogeochemical processes have the potential to significantly alter the dynamics of ecological communities. For tropical rain forests, these impacts may have important consequences for the long-term sustainable utilization of forest resources, and for the long-term conservation of biological diversity. However, until recently baseline information on tropical forests in Asia had not been systematically collected. In 1985, the Center for Tropical Forest Science - Arnold Arboretum Asia Program, a joint initiative of the Smithsonian Tropical Research Institute and Harvard University, embarked on an ambitious program to monitor representative samples of tropical rain forests in South and Southeast Asia. Seven large-scale research plots have been established in five countries. These plots together include more than a million trees comprising over 2,500 tree species. In this talk, the dynamics of several forests are compared to investigate whether there are region-wide directional changes in the dynamics of Asia's forests. The results are used to infer likely future changes in Asia's forests.

4.15.3. Ecological studies of Australian rainforests at CSIRO's Tropical Forest Research Centre

D. W. Hilbert, C. Margules, D. J. Metcalfe, D. Westcott; CSIRO Tropical Forest Research Centre, Atherton, Queensland, Australia.

The Tropical Forest Research Centre (TFRC) is centrally located within the Wet Tropics Bioregion which comprises the largest extent of Australia's tropical rainforests and associated tall open forests and swamp forests. There are approximately 30 staff and facilities to house both long and short-term students, visitors and collaborators. The centre has excellent computing, GIS and library services and includes an herbarium with the world's most comprehensive collection of Australia's tropical flora, and an arboretum containing c. 1500 trees. Ecological research includes

studies of the processes and consequences of seed dispersal, climate change impacts on forests and biodiversity, and the dynamics of rainforests. The TFRC also manages 20 long-term permanent plots which cover a wide range of forest types existing across different geological facies, temperature and rainfall regimes. The talk will summarise the key results in these areas. For example, our research has highlighted the great sensitivity of tropical highland species to future climate change. It will also identify some key gaps in our understanding and opportunities for future research.

4.15.4. Successional vegetation dynamics in the Caribbean lowlands of Costa Rica

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To examine successional changes in vegetation dynamics and species composition of woody vegetation, we conducted annual or sub-annual censuses from 1997-2004 in four 1-ha monitoring plots in secondary forests initially 12-25 yr old. Younger stands showed dramatic changes in size class distribution and species composition. Common tree species changed dramatically in abundance within plots, reflecting high mortality of early colonizing tree species and high rates of recruitment of shade-tolerant tree and palm species. Similar trends were observed for woody seedlings; shade-tolerant palm and canopy trees increased in species richness and relative abundance due to low mortality and disproportionate recruitment of tree and palm species. Shrub and liana species generally showed poor recruitment and high mortality and populations declined over time within and across sites. Species richness of trees and palms increased in all sites. Although chronosequence studies suggest that succession in tropical forests leads to convergence in species composition, these findings are not generally supported by long-term studies within individual sites.

4.15.5. "Tropenstation La Gamba" - a field station in the species-rich lowland rain forest of Piedras Blancas National Park, Costa Rica

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Inst. f. Botanik, Vienna, Austria.

The "Tropenstation La Gamba", Costa Rica, is an Austrian research station and education centre, whose aims are to contribute to the exploration and conservation of tropical forests in the neotropics. The station is situated in southeastern Costa Rica, 8°41'N - 83°13'W, at the western edge of the Piedras Blancas National Park. For detailed information see www.lagamba.at. The park area holds various types of rain forests which are among the most species-rich plant communities in Central America (up to 179 species per hectare). Special features include: • strong spatial structure at the landscape scale, that is providing numerous microhabitats and ecological niches • high annual precipitation (nearly 6000 mm), absence of a dry season • high edaphic heterogeneity and diversity of soil types • high vegetation dynamics. Analysis of the distribution patterns of the woody plants recorded in selected plots reveals a strong phytogeographical affinity to the Chocó region (coastal Colombia and adjacent Ecuador) in South America.

4.15.6. Tropical field stations and their role in scientific biodiversity research: the Kuala Belalong Field Studies Centre in Brunei (Borneo)

A. N. Muellner¹, C. M. Pannell², M. W. Chase¹;

¹Royal Botanic Gardens, Kew, Surrey, United Kingdom, ²University of Oxford, Oxford, United Kingdom.

The forests of Brunei are among the richest in the world and represent the greatest living natural resource of the country. About 80% of Brunei is under forest cover, the majority of which is undisturbed primary forest. The Kuala Belalong Field Studies Centre (KBFSC) was built in 1990 and served as a logistic base for the Brunei Rainforest Project 1991-1992, a collaborative effort between the University of Brunei Darussalam and the Royal Geographical Society as well as a number of associated institutions in the UK and other nations. Geographical and biological disciplines were brought together to describe climate, regional geology, landform processes and soils and the complexity of tropical rainforest biodiversity and ecology. This has set the course for continuing research, undergraduate training and school visits from all over the world. We present a two-year EU project on

biodiversity and conservation in Meliaceae (“mahogany” family) currently underway at the Royal Botanic Gardens, Kew and involving collaboration with the Forestry Department of the Ministry of Industry and Primary Resources in Brunei, the University of Brunei Darussalam and KBFSC.

4.15.7. The dynamics of a neotropical forest: results from a 25-year study

S. P. Hubbell;

University of Georgia, Athens, GA, United States.

For the past quarter century, we have been studying the dynamics of the neotropical moist forest on Barro Colorado Island (BCI), Panama, with the goal of testing the major hypotheses for the maintenance of alpha tree diversity in tropical rain forests. In 1980 we established a permanent 50 ha plot on BCI, in which all woody stems of free-standing trees and shrubs with a diameter of 1 cm or larger at breast height were measured, mapped, and identified to species, over 200,000 stems of more than 300 species. We have followed the individual fate of these plants for 25 years, recording growth, survival, and new recruitment. These data are supplemented with a long-term study of seed rain and seedling germination in the plot, and data on canopy height and gap dynamics. In this talk, we will summarize the principal findings concerning five major hypotheses for the maintenance of tree diversity in the BCI forest: the regeneration niche hypothesis, the intermediate disturbance hypothesis, the Janzen-Connell enemies hypothesis, the Chesson-Warner hypothesis, and the Hurr-Pacala recruitment limitation hypothesis.

4.16.1. The significance of the tyrolean iceman for the archaeobotany of Central Europe

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More than ten years of investigations on the find assemblage of the Tyrolean Iceman “Ötzi” reveal a great deal of information about the living and environment of this Neolithic man in the Alps. The palaeoethnobotany of his artefacts discloses a skilled person, well adapted to the alpine environment. Since the discovery of an arrow head in his left shoulder a strong evidence for a violent death is on hand, but still several questions are unanswered about his demise and deposition on the Tisenjoch in 3200 m altitude. Furthermore, besides the dispute about the Iceman’s personal fate, a discussion about the significance of the find assemblage for the Alpine Neolithic has started. At the moment only circum alpine settlements are well investigated and provide an analogy for the find. Archaeobotanical studies in the Alps have to be enhanced to assess the impact of the Iceman on the Alpine Neolithic.

4.16.2. Bryophytes and the iceman

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There are many thousands of fragments of bryophytes (mosses and liverworts) that have direct or indirect relevance to the life and times of the Iceman. The fragments range from pieces less than 1mm long (half a leaf of *Antitrichia curtispindula*) to a mass of *Neckera complanata* of leafy stems 60 mm long. Totalling more than 80 species, the fragments have been: 1. Recovered from the mineral sediments that partly filled the hollow in which the Iceman lay and from mammalian droppings found in the sediments. 2. Washed from his clothes and gear. 3. Found in preparations of his intestinal contents made for pollen counting. The interpretations of such abundant data have great importance for the elucidation of the palaeoclimate and vegetation, for the understanding of the usefulness of mosses for prehistoric people and for the deduction of the Iceman’s provenance and the details of his last journey.

4.16.3. The plant remains from the Iceman’s find spot - new results on the glacier mummy’s environment

A. G. Heiss, K. Oeggl;

University of Innsbruck, Institute of Botany, Innsbruck, Austria.

Numerous plant remains recovered from the Iceman’s find spot in the Ötztal during the 1991 campaign were analysed. The material, embedded in the glacier ice for more than 5,000 years, was in an excellent state of preservation. The identified macro remains derived from different sources: - fragments of the Iceman’s artefacts, such as slivers of his wooden tools and weapons - plants

adhering to the Iceman’s clothes and grass mat - local alpine vegetation at or near the discovery site - plant remains transported to the site by other human/animal activity, or by wind. The results provide further information on ecosystems and climate in the Eastern Alps during the Neolithic. The presence of alpine species from crevices and snow pocket plant communities may indicate a climate slightly milder in the Neolithic than today, supporting multi-proxy data (e.g. Patzelt 2000) on a reduced glacier extent during the Iceman’s lifetime. Furthermore, the remarkably even distribution of plant remains across the samples suggests one or more phases of less ice at the discovery site in the past. During these the ice in the gully had thawed, and the find assemblage had floated in the melting water.

4.16.4. Origin and seasonality of subfossil caprine dung from the iceman’s discovery site (Eastern Alps)

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Macrofossil and pollen analyses of 103 caprine dung pellets from the Iceman’s discovery site at 3,200 m a.s.l. were conducted to ascertain if some derived from livestock. Such an evidence would confirm Neolithic transhumance for the inner alpine area. The proof should result from the analysis of the animal’s diet: animals grazed in anthropogenic habitats of low altitudes would indicate the presence of livestock, whereas diet consumed only in the alpine grasslands argues for game. Analogues were established by collecting modern pollen surface samples along an altitudinal transect in the area and modern caprine faeces at the discovery site over a vegetation period. Fruits and epiderms of alpine herbs occurred frequently in the subfossil samples and indicate grazing in alpine grasslands. This conclusion is supported by the ratio of arboreal to non arboreal pollen in the subfossil dung and by the comparison of the dung pollen samples with recent surface samples. The calibration with the modern dung pollen spectra reveals that the subfossil dung was deposited at the Tisenjoch in late summer.

4.16.5. Plant economy of the late 4th millennium BC cal in the Northern Alpine Foreland

S. Jacomet;

IPAS, University of Basel, Basel, Switzerland.

In Neolithic Lakeshore settlements, millions of subfossil plant remains are found, and therefore a detailed reconstruction of the economy is possible. Of the time of the Iceman, however, not very much such settlements are preserved. There are a few exceptions like the site Arbon Bleiche 3 at Lake Constance (Canton of Thurgau, Switzerland), who lasted only 15 years, from 3384 to 3370 BC (dendrochronological dating). The cultural layer of that settlement was evaluated during a multidisciplinary research project, founded by the Swiss National Foundation. The main cultivated plants were a tetraploid naked wheat, emmer and a multi-rowed barley. Beside of that, flax and opium poppy played an important role. Agricultural practices could be reconstructed with the help of weeds. Beside agriculture, gathering was very important. Botanical investigations of the ruminant dung allowed to reconstruct in detail the keeping of sheep/goat and cattle. In the lecture the most important results of the Arbon Bleiche 3 site will be presented, and compared with the spectra of other settlements of the second half of the 4th millennium BC cal.

4.16.6. Late neolithic agriculture in eastern Austria - archaeobotanical results from sites of the Jevišovice culture (3000 BC).

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Charred plant remains from huts destroyed by fire have provided first informations on late neolithic agriculture in eastern Austria. The two Jevišovice sites investigated show us that farmers around 3000 BC were relying on the use of *Hordeum vulgare* and *Triticum monococcum* as cereals with *T. dicoccum* and *Panicum miliaceum* as additional crops. *Pisum sativum* and *Lens culinaris* provided vegetable protein, *Papaver somniferum* and *Linum usitatissimum* vegetable fat. The record of *Panicum miliaceum* documents a remarkably early westward spread of this species. Among the wild plants, the occurrence of *Stipa* sp., *Teucrium chamaedrys*, *Asperula cynanchica* and *Plantago media* point to the presence of dry steppic grassland in the vicinity of the settlements, which was

probably used for grazing. Woodland species were mainly represented by fruit-bearing plants growing in clearings and forest edges, reflecting both the purposeful collection of wild fruits and the growing human impact on the vegetation.

4.16.7. Diet of pile dwellers and formation of the cultural landscape south of the Alps: a case study from Slovenia

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Analyses of plant remains from the Eneolithic pile dwelling Hocevarica on Ljubljana Moor, Slovenia, were performed. Seeds, fruits, macroscopic charcoal and pollen from the cultural layer were analysed. Numerous remains of nuts and berries, especially of *Quercus sp.*, *Cornus mas*, *Rubus fruticosus* and *Corylus avellana* demonstrate that gathering presented an important part in the economy of early settlers south of the Alps. In addition, the pollen of Cerealia, charred grains of cultivated barley (*Hordeum vulgare*) and wheat (*Triticum monococcum*, *T. dicoccum ssp. turgidum*) and poppy seeds (*Papaver somniferum*) and seeds of Chenopodiaceae indicate early cultivation in the area. Data from Hocevarica further suggest that cleared Neolithic land was used for agriculture and pasturing, and that different wood was cut for construction and for firewood. In comparison to rather similar paleobotanical evidence from Northern Alpine lake dwelling areas, a few different taxa (e.g. *Lathyrus sativus*, *Vicia sp.*) were identified. Grape seeds (*Vitis vinifera ssp. sylvestris*), dated to about 3600-3500 cal B.C., represent the oldest remains of grapevine from Slovenia.

4.17.1. Biochemical basis for quality differences of green coffees

D. Selmar;

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Green coffee is obtained by either wet or dry processing of coffee fruits (*Coffea arabica*), i.e. the coffee cherries are mechanically depulped, then the residue of the pulp is degraded by fermentation and the seeds are dried, or the entire coffee cherries are dried directly. Wet processed green coffees reveal a higher quality than dried processed ones. Up to recently, the basic causes for these quality differences have not really been understood. Due to new biochemical approaches and considering that freshly harvested green coffee seeds represent viable organisms that are able to respond to their environment, a novel perspective of interference of quality with an active seed metabolism was elaborated. Two major metabolic processes are executed within the coffee seeds during post harvest treatment: germination and a stress metabolism. Their extent depends on the mode of processing and thereby determines coffee quality. These considerations are the basis to understand the generation of related quality differences and can be used to work out feasible approaches to alter the ancillary conditions of coffee processings and thus to enhance coffee quality.

4.17.2. Medicinal and aromatic plants in Bangladesh: ethnobotanical survey and identification of endangered species in the barind tract

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An ethnobotanical survey has been made in the Barind tract to address the endangered medicinal plants still existing over the area. Four thousand folk medicinal practitioners were interviewed under prescribed questionnaires enquiring the uses of medicinal plants and their availability. Two hundred plants belonging to 56 families were identified, to have important medicinal values, were analyzed and categorized according to the respondent percentage. The degree of threat to the individual taxon was distributed on respondent performance following the guidelines of IUCN. Nineteen medicinal plants named as *Withania somnifera*, *Aristolochia indica*, *Hemidesmus indicus*, *Mimosa pudica*, *Curculigo orchoides*, *Ipomoea mauritiana*, *Leea microphylla*, *Gloriosa superba*, *Scindapsus officinalis*, *Cassia alata*, *Tinospora cordifolia*, *Plumbago zeylanica*, *Vitex negundo*, *Asparagus recemosus*, *Andrographis paniculata*, *Rauvolfia serpentina*, *Rauvolfia tetraphylla*, *Smilax zeylanica* and *Abrus precatorius* were determined as the endangered medicinal plants falling the highest rating score of 90% - 100%.

4.17.3. Cocoa seed fermentation: Factors influencing quality - a survey

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The fermentation process of cocoa beans is of paramount importance for the formation of cocoa aroma. There are over 400 different aroma related compounds that have been identified in fermented, dried and roasted cocoa beans. The quality and quantity of these substances are influenced by different parameters such as: the conditions of fermentation and drying, genetic background as well as genotype x environment interactions (e.g. the temperature regime during the ripening process). However, without the fermentation process, no cocoa aroma precursors are formed. This paper will discuss recent findings on the influence of pulp condition, the importance of testa morphology on acetic acid diffusion into the cotyledons, and tissue acidification. The importance and influence of the seed testa on transport processes and its impact on aroma formation as it varies with the genotype will be a central point of discussion. The data presented will support the hypothesis that maternally influenced aroma precursor formation is due to biochemical precursors of the fresh seeds and on structural parameters influencing the kinetics of import and export of solutes during fermentation.

4.17.4. Could semi-desert plants used for production of bio-pharmaceuticals and needed secondary metabolite?

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Considering the climate condition there is a wide region of semi desert and desert area with variety of plants that are living there. Analytical and industrial methods for extraction and identification of natural products from semi desert plant now widely used. This make a huge potential for using of these plant as a factory for production of Biopharmaceuticals. The central Iranian sector hosts the most typical vegetation of Iran's steppe and desert plant. From these, *Atriplex* and *Artemisia* cover wide area in this region. In this project we studied, introduction of Human growth hormone (hGH) by agro bacterium to these semi-desert plant (*Atriplex lentiformis*) by transient (Vacuum agro infiltration) and expression analysis of this pharmaceutical gene was done. The produced hormone was analysed by ELISA, Western and RIA assay. The produced hormone was Bioactive and has a Band in 22 Kd in western analysis.

4.17.5. Chemical relevance of the epicuticular wax of mango fruit

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High levels of solarisation in South Africa affect the development and ripening of mango fruit (*Mangifera indica* L.) during commercial production. However, the naturally dense and complex epicuticular fruit wax is an effective protective barrier. This wax occurs in two chemically identifiable layers that were characterised with Raman and IR spectroscopy. The layers develop in distinguishable stages with increasing complexity in both constituency and architecture, as found with SEM. Lenticels on the fruit surface are also lined by some of the wax fractions, with the quantity and structure dependent on the mango cultivar. A correlation was found between the amount of wax in lenticels and cultivar susceptibility to lenticel discolouration, an economically unacceptable condition. Although the trigger for development of the discolouration is uncertain, HPLC results and TEM have indicated intracellular and wall bound phenolics as the cause of visible symptoms. Fluorescence microscopy has indicated that wax in less susceptible lenticels contains phenolics. Current studies are investigating volatile compounds as a possible trigger mechanism.

4.17.6. Chemical constituents of *Salacia reticulata*

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Kothala himbutu (*Salacia reticulata*) is woody liana, distributed in Sri Lanka and south India. The stem tissue is used for a traditional herbal medicine in Sri Lanka. Since hot water extractions from the stem reduce sugar content in blood, being effective for diabetes, the plant is valuable commercially. Mangiferin, isomangiferin, and (-)-4'-O-methyl-epi-gallocatechin were isolated and identified by spectroscopic analysis. HPLC analyses clarified the fact that almost all these compounds have been lost during the traditional drying process of the raw materials. The extraction efficiency is extremely low when the extractions are obtained after drying the material, leading to economical loss in commercial activities. Chemical constituents of *Salacia reticulata* and the economical loss by the change of the amounts of the chemical constituents during drying the raw materials are discussed.

5.1.1. Studying abiotic stress in plants: from gene networks to desert plants.

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Within their natural habitat plants are subjected to a combination of different abiotic stresses. Key to plant acclimation to these conditions is a network of pathways that enables plants to mount a rapid and flexible defense response. We are using a combination of transcriptome, metabolome, and proteome profiling to study how plants acclimate to a combination of drought and heat stress. A systematic approach of characterizing mutants and transgenic plants altered in the expression of key defense and regulatory genes is in progress. Our findings suggest that the response of plants to a combination of two different abiotic stresses is distinct from the response of plants to each of the different stresses applied individually. To complement our analysis of lab-grown plants, a field study of desert plants is being conducted to test whether some of the hypotheses generated in the lab hold true for desert plants growing within their natural habitat.

5.1.2. Gene expression profiles during salt stress in poplar

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Among tree species, the genus *Populus* harbours a huge variety of ecotypes with different degrees of stress resistance. The species *Populus euphratica* has the ability to cope with saline environments. In order to identify genes regulated under salt stress we performed microarrays studies with arrays harbouring a unigene set (7745 ESTs) from *Populus euphratica*. These arrays were hybridized with cDNA from leaves resp. roots from salt treated *P. euphratica* plants and controls without stress treatment. Transcript levels of 135 genes were altered significantly, 86 in roots and 55 in leaves, 5 in both tissues. Based on these data we present candidate genes for the salt tolerance in poplar and formulate hypotheses about the relative importance of various physiological processes during salt stress. This work was performed with financial support of the DFG to Poplar Research Group Germany.

5.1.3. Nitric oxide induces callose formation in plant cells

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If onion inner epidermal cells are incubated in a Na-nitroprusside solution (as NO-Donor), callose deposition on cell walls is observed. First small plaques are seen after 5 - 6 hours. Callose deposition continues, after 20 - 24 hours one or more, often nucleus-sized, lens shaped bodies are seen in each cell. The optimal concentration of Na-nitroprusside lies between 1.0 and 0.5 mM. The callose-inducing effect of NO is inhibited by the nitric oxide scavenger methylene blue, the calcium channels antagonists nifedipine or ruthenium red, and the non-hydrolyzable guanosine-nucleotide analogs GTP- γ -S and GDP- γ -S. The phosphodiesterase inhibitor isobutyl-methylxanthine enhanced callose formation. It is concluded, that downstream signalling involves formation of cGMP as a second messenger and activation of ion channels. Callose formation seems to be a defence

mechanism against the disturbance of the cytoplasmic Ca²⁺-balance.

5.1.4. Nitric oxide - key component of plant innate immunity

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As recently as 1987, NO was widely considered to be just an air pollutant and a toxic gas. However, by 1988, the evidence was overwhelming that NO was an integral part of normal physiological function in animals. Subsequently, it was realized that plants not only respond to airborne NO but that NO was a novel messenger in plant defence signalling. Not only is it important in pathological processes but it is also a crucial player in the regulation of physiological processes including seed germination and stomatal closure. The observation that NO regulates the expression of many genes suggests that it elicits numerous physiological processes in plants. The evidence presented to date suggests that crucial players in animal NO signalling also operate in plants. These include major direct targets of NO through S-nitrosylation, and the second messengers cGMP and cADPR. The major challenges ahead are to determine which of the NO-producing enzymes in plants participates in a particular physiological process, and how the correct specific response is evoked despite shared use of the NO signal.

5.1.5. Regulation of plants by herbicide safeners

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Safeners are agrochemicals which enhance tolerance to herbicides in cereal crops by increasing the expression of protective detoxifying enzymes. The proteins concerned include the cytochrome P450 mixed-function oxidases, glutathione transferases, glucosyltransferases and ATP-binding cassette transporters. Effectively safeners are causing a specific stress response representing a co-ordinated induction of the first three phases of xenobiotic detoxification. It has recently been shown that similar enzyme induction is also shown in dicots such as *Arabidopsis thaliana* and soybean even though herbicide-safening is restricted to monocot crops. This suggests that safeners are interfering with a conserved signalling pathway of unknown function which regulates specific branches of primary and secondary metabolism in different plants. Using a combination of proteomics and metabolite profiling, progress toward defining these changes in metabolism will be presented. The objective of these studies is to develop a new paradigm as to the functions of the safening response in plants and to ultimately use this trait in biotechnological applications.

5.1.6. Lupins as model plant for translocation and long distance signalling

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As a potential model for studies of translocation of assimilates and nutrients, as well as signalling molecules involved in regulation of plant development, lupins are unsurpassed. From a number of species in the Genus, including *Lupinus angustifolius*, *L. albus* and *L. mutabilis*, prolonged phloem exudation permits direct collection of translocated sieve tube contents in quantities sufficient for detailed transcriptomic, proteomic and metabolomic analysis. A sound physiological basis has been established that relates xylem and phloem fluxes of solutes containing C and N, inorganic nutrients, plant growth regulators (ABA and cytokinins) and alkaloids to establishment of source/sink features and the functioning of specific organs. In addition to providing the vascular network for translocation of assimilates, phloem also provides a pathway for long-distance signalling. As well as the known plant hormones phloem exudates contain at least 200 proteins and peptides, mRNA, si and miRNAs. The potential for exploiting these features in lupin to document phloem mobile regulatory RNA signals and RNA-mediated signalling processes is significant.

5.1.7. Evaluation of natural and synthetic stimulants of plant immunity

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Induction of local defence as well as systemic resistance of plants is associated with transcriptional reprogramming. Here we report on defence gene induction by natural and synthetic stimulants of plant immunity. Gene expression changes in *Arabidopsis thaliana* were monitored in response to several plant immunity stimulants (plant activators) using an application based array representing about 750 genes involved in several aspects of plant defence and/or plant stress. The commercial plant activators Bio-S®, Neudo-Vital® and PRORADIX® have been shown to induce systemic resistance. Here, Neudo-Vital®, PRORADIX®, and Bio-S® treatment induced different patterns of salicylic acid (SA) and jasmonic acid (JA) accumulation. Rather than simply mimicking one of the known defence pathways induced by SA or JA, the response to the plant activators showed aspects of both major defence systems. A general feature was the transient activation of JA-dependent responses, combined with a much more sustained, SA-associated defence gene induction.

5.2.1. Transgenic plastids as expression factories in biotechnology

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There are considerable attractions of the plastid genome as a target for the expression of foreign genes, including (i) high level foreign protein accumulation, (ii) absence of epigenetic effects and gene silencing mechanisms, (iii) simultaneous expression of several genes linked together in an operon and (iv) lack of pollen transmission of the transgene due to maternal plastid inheritance in most crops, allaying concerns over environmental consequences of GM crop cultivation (Bock, *J. Mol. Biol.*, 312, 425-438; Khan and Bock, *Trends Biotechnol.*, 22, 311-318). We have recently developed a first plastid transformation system for a food crop with an edible fruit (Ruf et al., *Nature Biotechnol.*, 19, 870-875). Transplastomic tomato plants are fertile and express foreign genes to high levels even in non-green plastids (chromoplasts) of the ripe tomato fruit. This system now paves the way to safe metabolic engineering and efficient production of nutraceuticals and biopharmaceuticals in consumable plant organs. Technological aspects, new tools for transgene expression in plastids as well as selected applications will be discussed.

5.2.2. Next generation plastid transformation technology

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Plastid transformation presents an attractive platform for expression of recombinant proteins due to the potential of high yield, the avoidance of transgene spread via pollen and the precise insertion of transgenes by homologous recombination. We have developed expression control elements, based on the insertion of transgenes into existing plastid operons, on use of heterologous expression control elements and on N-terminal fusions. Efficiently expressed recombinant proteins include somatotropin, interferon alpha and phenyl ammonia lyase (PAL). A novel marker gene, *aphA6*, was established for selection of transplastomic lines. Use of a visible marker, based on the reactivation of artificially created mutants, facilitates selection of homoplastomic transformants. Novel transformation vectors, exploiting co-integrates, which are formed during plastid transformation, lead to automatic removal of selection markers. Using lac repressor/operator elements controlled expression of plastid transgenes can be achieved through direct induction of plastid gene transcription via an externally applied chemical signal such as IPTG.

5.2.3. Site-specific recombinases for engineering the plastid genome of higher plants

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We developed new tools to manipulate the tobacco plastid genome with phage site-specific recombinases. CRE is used for targeted excision of marker genes flanked by lox sites (floxed) after expressing the plastid-targeted recombinase from a nuclear gene. The gene encoding CRE may be stably integrated in the nucleus, or expressed transiently after Agrobacterium infiltration. Transient CRE expression significantly shortens the time required to obtain a marker-free transplastomic plant and avoids going through a seed progeny to segregate away the integrated nuclear cre gene. Floxed marker genes are now incorporated in new plastid vectors

so that plastid transformation and marker gene removal can be executed efficiently. INT, a second site-specific recombinase, is used to facilitate integration of transforming DNA by recombination between an attB site integrated in the plastid genome and an attP site in the vector. We now explore the feasibility of employing INT to overcome low plastid transformation efficiency in *Arabidopsis thaliana*, a species recalcitrant to plastid transformation.

5.2.4. Plastid transformation in the moss *Physcomitrella patens*

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The moss *Physcomitrella patens* is widely used as a model plant because it has the highest known homologous recombination ability among land plants. We have recently succeeded in plastid transformation and demonstrated that the moss arginine tRNA-CCG gene, *trnR-CCG*, is not essential for plastid function (Sugiura and Sugita, 2004). For the targeted disruption of *trnR-CCG*, the gene was replaced with a selectable spectinomycin (spec)-resistance (*aadA*) gene cassette from pZS197 (Svab and Maliga, 1993) on a plastid gene fragment in the plasmid. The transforming plasmid DNA digests were introduced into protonema protoplasts in the presence of polyethylene glycol. The correct insertion of *aadA* into the targeted plastid genome in transformants was verified by Southern blot and PCR analyses. The spec-resistant phenotype of the transformants was confirmed to be inherited via the plastid genome. Thus, plastid transformation successfully achieved targeted gene-disruption in the moss. This will allow investigation of the molecular basis of the regulation of plastid gene expression and cost-effective production of proteins in the moss plastids.

5.2.5. Ethanol-inducible transcription of the *Ralstonia eutropha* *phb* operon in transplastomic tobacco and formation of polyhydroxybutyric acid (PHB)

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Constitutive transgene expression can be accompanied by deleterious effects if gene products are harmful to the transformed plant. Constraints like growth inhibition and male sterility have been observed in plastid transformants containing the *phb* operon encoding the genes required for the production of the polyester polyhydroxybutyric acid (PHB). In order to induce PHB synthesis in tobacco in a well-timed manner, we have constructed an inducible system to regulate transcription of the *phb* operon in plastids. This system consists of a nuclear located, ethanol inducible T7RNA polymerase which is targeted to plastids harboring the *phb* operon under control of T7 regulatory elements. Following treatment with ethanol, moderate induction of PHB synthesis was found. PHB amounts reached up to 1383 ppm in dry weight and an overall background activity of 171 ppm was measured in uninduced tissues. Without ethanol application the plants developed flowers and fertile seeds. Thus, the main problem of inhibitory transgene expression was solved. Our results show that this inducible system could serve as an alternative to constitutive expression of plastid transgenes.

5.2.6. Bacteriophage T7 RNA polymerase directed regulated expression of foreign genes transformed into tobacco plastid genome

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There is an increasing interest to express genes coding for subunit vaccines in traditionally non-medicinal plants. Such plant expressed vaccines were found to be immunogenic when administered through injection or orally. However, a critical factor that determine the success of this approach depend on high level expression of antigens in plants. We have transformed tobacco plants to overexpress Hepatitis B surface antigen (HBsAg) in chloroplasts. However, overexpression of the antigen had a profound effect on the normal growth of transplastomic plants. Such a problem was addressed through the development of an

inducible expression system based on the transcription of HBsAg by T7 RNA polymerase. This was achieved by introducing HBsAg under T7-expression signals into plastid genome followed by the transformation of tetR under CaMV 35S promoter into nuclear genome. Again, the same plant was transformed with a modified T7 RNA polymerase fused with the *rbcl* transit peptide sequence placed under CaMV 35S promoter containing tetR binding sites into nuclear genome. Results from the new expression system will be presented.

5.2.7. Plastid transformation for the production of pharmaceutical proteins in tobacco

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Plastid transformation has great potential for the high level production of foreign proteins, particularly for pharmaceutical proteins that might be used for oral vaccines. We have used the green fluorescent protein reporter gene to compare the effects of different promoters and 3' untranslated regions (UTRs) on expression in tobacco chloroplasts. Chimeric gene constructs in a pZS197-based vector have been introduced into the *rbcl-accD* intergenic region of the tobacco plastid genome following microprojectile bombardment. The tobacco plastid *rrn* promoter with the *rbcl* 5' UTR gave higher amounts of *gfp* transcripts and GFP than the tobacco *psbA* promoter and 5' UTR or the *E. coli trc* promoter. Transcripts containing the *E. coli rrmB* 3' UTR were more stable than *gfp* transcripts with the tobacco *rbcl*, *psbA*, *rpoA* or *petD* 3'UTRs. The tobacco plastid *rrn* promoter and *E. coli rrmB* 3' UTR have been used for the expression of rotavirus and HIV genes in tobacco chloroplasts. Rotavirus VP6 protein was produced at ~3% total soluble protein in young leaves, but disappeared as the leaves aged. Accumulation of rotavirus VP6 appears to be limited by protein stability in older leaves.

5.3.1. Models of phyllotaxis

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A major determinant of plant architecture is the arrangement of leaves and flowers around the stem, known as phyllotaxis. Spiral phyllotaxis is of particular interest, because the divergence angle between successive leaves approaches the golden ratio of 137.5°, and the spiral arrangements are characterized by Fibonacci numbers. Although mathematical models can recreate phyllotactic patterns, experimental support for them has largely been lacking. I will present experiments that establish the plant hormone auxin as a central regulator of phyllotaxis. These experiments provide the basis for biologically meaningful models that can guide the design of new experiments. Ultimately, models should account for all aspects of phyllotaxis, for its reiterative nature, regularity and stability, as well as for the transitions between phyllotactic systems.

5.3.2. The Arabidopsis elch mutant: Part of a new ubiquitin-dependent protein degradation pathway in plants?

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In a forward genetic screen for trichome mutants we have identified the Arabidopsis *elch* mutant. Mutant plants have multinucleate trichomes with multiple stems. The cell-cycle defect is not trichome-specific but also affects other cell types as pavement cells or hypocotyl cells. Cloning of the *ELCH* gene revealed homology to the yeast *vps23* gene, which is involved in sorting of monoubiquitylated proteins into the inner vesicles of multivesicular bodies resulting in delivery of the cargo to the lumen of the yeast vacuole, where it is subsequently degraded. As its yeast counterpart, the *ELCH* protein can bind ubiquitin. In yeast, *vps23* acts in concert with a large number of other proteins forming three complexes termed ESCRT I-III. Homologs of all ESCRT components can be found in the Arabidopsis genome. We performed a Two Hybrid analysis of the Arabidopsis ESCRT homologs and could establish an interaction network similar to the one existing in yeast involving *ELCH* and the other Arabidopsis ESCRT candidates. Taken together, our data suggest that a monoubiquitylation-dependent protein sorting pathway exists in plants that is involved in the regulation of the cell cycle.

5.3.3. 100 years of flowering: the pros and cons of dominant concepts

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In the field of flowering, research has progressed along lines determined by a small number of dominant concepts. 'Turning points' in the field are the times when these concepts have been adopted by the research community. In this paper I shall describe these dominant concepts: where they arise, what they mean, and how they set the research agenda. I shall also describe two aspects of flowering that demonstrate how progress is made by combining data derived from different conceptual eras. One example connects floral signalling and the genetics of flowering time; the other concerns phase change. In the case of phase change, meristem fate is controlled both by the temporal expression pattern of key genes within the meristem, and by signals arriving from other parts of the plant. Its full significance will emerge as the topic of perennial flowering is explored in future research. The conclusion is that concepts are useful but if they are too dominant they inhibit not just research progress, but also synthesis of knowledge.

5.3.4. Auxin gradients as common module for plant patterning

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Plant hormone auxin is a prominent intercellular signal in plants. Directional, active, cell-to-cell transport over short distances mediates local, differential auxin distributions (gradients), required for various patterning processes, including apical-basal axis formation and organogenesis. Also growth responses to environmental cues such as light or gravity utilize a similar mechanism involving auxin asymmetric auxin distribution. Differentially expressed auxin transport facilitators of the PIN family, each with specific polar, subcellular localization form a network for auxin distribution and formation of these local gradients. The activity of PIN proteins can be regulated at the single cell level by changes in their vesicle trafficking-dependent polar targeting in response to developmental and environmental cues. Thus, this auxin distribution network, whose directional throughput is modulated by both endogenous and exogenous signals, provides, by means of mediating auxin fluxes and creating local gradients, a common mechanism for the plasticity and adaptability of plant development.

5.3.5. MicroRNA regulation of gene expression during Arabidopsis leaf development

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Organisms are patterned by the complex regulation of gene expression and activity during development. A recently discovered class of gene regulator, the microRNAs (miRNAs), consists of ~22-nucleotide noncoding RNAs that regulate gene expression by directing mRNA degradation or inhibiting productive translation. We discovered miRNAs in plants and computationally predicted their targets. Two related miRNAs, miR165 and miR166, regulate the activity of HD-ZIP III transcription factors such as *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*) in patterning the adaxial and abaxial domains of the Arabidopsis leaf. MicroRNAs may regulate HD-ZIP III gene expression at multiple levels; miRNAs can direct degradation of the *PHB* mRNA but also influence the methylation state of the *PHB* and *PHV* genomic loci. We are currently exploring the roles of transcriptional and post-transcriptional regulation of HD-ZIP III gene expression in the developing leaf primordium.

5.3.6. Physiological and molecular evidence for Darwin's "brain hypothesis" of the plant root cap: the case for maize seedlings

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Plant roots cope during their entire life with changing surroundings concerning water, nutrients, mechanical impedance, temperature, and pathogens. They perceive and process external and internal

signals, thereby enabling the plant to develop optimally. Earlier studies demonstrated ethylene to interfere with root elongation growth and root hair formation. Here we investigated the relevance of the root cap in maize seedlings for ethylene-dependent growth and -root hair formation, also employing RT-PCR and GC-ethylene measurements. Cap removal prevents seedling roots to respond towards ethylene as well as towards its precursor aminocyclopropane-1-carboxylic acid (ACC). We are trying to elucidate the causal scenarios and provide evidence that root caps operate as command centres for the development of the root also affecting the shoot. They coordinate ethylene action on root-growth and -hair formation. Root caps apparently play a crucial role in determining the developmental fate of the plant by intervening with the expression and realization of signals in remote tissues; - they therefore appear to “act like the brain of one of the lower animal” (Darwin)

5.3.7. Revealing the genetic mechanisms regulating the vascular cambium and secondary growth in woody plants

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Recent studies are defining the genetic mechanisms regulating secondary growth (the radial growth of woody stems). The emerging paradigm suggests that many of the key genes and mechanisms that regulate the meristematic “stem cells” of the shoot apical meristem also function in the cambium region of woody stems. I present experiments describing the cloning and characterization of the *Populus* ortholog of two key regulators of the shoot apical meristem, *SHOOTMERISTEMLESS* (*STM*) and *BREVIPEDICELLUS* (*BP*, also known as *KNAT1*). I show that the *Populus* orthologs of *STM* and *BP* are expressed in the cambium region, and that misexpression of these genes result in phenotypes with defects in secondary growth and wood formation. These results are discussed within the context of the evolution of secondary growth and the relationships of woody plants to one another and to herbaceous plants.

5.4.1. Observations on the History and Future of Liana Biology

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Although lianas play critical roles in the ecology and management of many forests, the firm foundation for liana biology provided by Darwin, Schenk, and others in the 19th Century was only sporadically reinforced by researchers until relatively recently. Although recognition of liana contributions to forest structure, composition, dynamics, and productivity has recently increased, interspecific differences among lianas are seldom reflected in community-level studies. Similarly, while interesting physiological features of climbing plants have been revealed, scaling up from autecology to community ecology remains challenging. We also still struggle to predict where lianas will proliferate and for how long they will persist, deficiencies in our knowledge that frustrate simulation modelers who recognize that forests are more than just trees. We also need to grapple with the fact that lianas are not restricted to growing upright, which makes them challenging to map, and have roots that spread more widely, grow more deeply, and colonize resource-rich patches more rapidly than those of the trees with which they so effectively compete.

5.4.2. Climbing habits in palms: South East Asia versus South America

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Climbing growth forms have appeared several times during palm evolution in at least three out of six subfamilies. With about 650 climbing species (22 % of all palms), the climbing habit is a successful growth form in the family. Dicotyledonous lianas can show extreme changes of mechanical properties during development, often due to modifications of cambial activity. This has been reported to reduce mechanical stresses resulting from swaying or fall from the support. Without secondary growth, climbing palms must retain part of the primary vascular system viable throughout their life history. This might appear to be a significant developmental constraint. It is of much interest to know how climbing palms, often with stem diameters, heights and

lengths comparable to dicotyledonous lianas, can maintain a scandent habit without secondary growth and specialized mechanical and wound-healing properties. This study focuses on the subfamilies Arecoideae (*Desmoncus*, South America) and Calamoideae (*Calamus*, South East Asia) with the objective of highlighting whether diverse origins of climbing in the family involve different “scandent strategies”.

5.4.3. Evolution of mechanical innovations and self repair in climbing plants

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Climbing plants have attachment organs for support and may rely on a stiff outer cortex as the main contributor for flexural stiffness of their axes. This tissue allows both stems and branches to have a long “searcher phase” and support by interlocking with other stems. However, during secondary growth the expansion of tissues can cause significant stresses and strains in cortical tissues. *Calamopitys* sp.(340 Myr) has a large parenchymatous inner cortex that can compensate these deformations so that the outer hypoderm remains intact and flexural stiffness is not reduced by secondary growth. In *Lyginopteris oldhamia* (300 Myr), the outer cortex also permits a relatively long stiff phase, which involved some degree of self-repair, but secondary growth eventually caused sloughing of the hypoderm marking a significant drop in stiffness. This development represented an improvement for the ability of the stem to resist damage via high bending and torsion stresses. Some recent lianas such as *Aristolochia macrophylla* have further evolved repair mechanisms for hypodermal steromes, enabling these plants to actively readjust their mechanical properties.

5.4.4. Secondary growth and formation of included phloem in stem of *Salacia reticulata* wight.

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Samples of stems of *Salacia reticulata* were obtained at several locations in Sri Lanka, and studied by conventional light or confocal microscopy (CLSM) or SEM. The formation of successive cambia and ‘included phloem’ were analyzed in series of cross sections along portions of stems. When a stem reaches one-two centimeters in diameter, some segments of cambium cease formation of xylem and grooved xylem boundary is formed. At a further stage, a ‘successive cambial layer’ forms in the middle portion of cortex. The ‘new cambium’ produces xylem inwards and phloem outwards that separate the bark into ‘outer bark’ and ‘included phloem’. The ‘included phloem’ consists of the previously formed phloem and cortical cells that have remained at the inner side of the new cambium. Successive cambia and alternating rings of included phloem and xylem are formed during the further growth of the stem. The rings of included phloem are connected with each other and the outer phloem at younger parts of the stem. The width of outer bark remains relatively constant during the secondary thickening of stem due to regular formation of successive cambia.

5.4.5. The hydraulics and wound repair of lianas

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Liana stems tend to have long and wide vessels and, relative to the leaf area they supply, narrow stems. Compared to free-standing growth forms, lianas have less requirements for mechanical tissue and their stems often contain relatively large amounts of unligified parenchyma and phloem tissue. As a result, liana stems are flexible and better able to regenerate if damaged compared to trees or shrubs. Although some lianas have positive root pressures that can serve to refill embolized vessels, most liana species do not. When hybrid poplar trees were staked, they tended to form narrower stems and wider vessels than in mechanically flexed controls. The staked stems have higher specific conductivity and lower flexural stiffness than controls. However, contrary to results reported for the liana *Toxicodendron diversilobium*, in the case of hybrid poplars staked plants had a greater Modulus of Elasticity than in flexed controls; flexing of poplar stems increased the amount of xylem but reduced the lignin content. The evolution of the liana habit may include fundamental

changes in gravitropic and thigmomorphic responses, especially with regard to lignin deposition.

5.4.6. Light and development in climbing plants

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The neotropical species *Croton nuntians* occurs as juvenile, freestanding growth phases and as adult climbing growth phases. When plants of *C. nuntians* start to climb they show an accelerated growth in length, leading to the formation of long, slender and unstable stems which get entangled in the surrounding vegetation. Cross-sections of climbing stems show a transition from a dense wood type formed in early growth phases to a very different one with large vessels, as reported for many other tropical lianas. In this species the transition to the formation of the flexible wood type with large vessels does not take place simultaneously with the shift to the climbing growth phase. Plants continue to form the dense wood type for at least half a year after transition to the climbing growth phase. Light availability has a strong influence on growth, stem and wood anatomy in *C. nuntians*, but cannot be considered as the exclusive factor triggering the shift from the freestanding to the climbing growth phase. Experiments with climbing species of temperate zones have shown that a combination of shade and support has a significant influence on the growth in length of tested species.

5.4.7. Lianas and tropical forestry

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Many tropical forests have been repeatedly logged over short felling cycles leading to a substantial loss in biomass and accordingly have given rise to a very open forest structure often aggravated by fires which penetrated them occasionally. Lianas often become gregarious having an advantage over tree seedlings and saplings as lianas hardly have to invest in supporting tissue and so can outcompete the former. Trees harvested often are infested by multiple large sized lianas causing large felling gaps, additional damage to surrounding trees and a deviation of the predicted felling direction. Not surprisingly, forest managers often see lianas as a nuisance and cutting of lianas prior to or after logging is often prescribed to reduce felling damage. Forest fires and liana cutting interventions both seem to have a long lasting effect on the liana populations in fragmented forests. However, on the longer term the numbers do not matter but the species diversity. In this presentation we will highlight some of the major aspects, illustrate them with examples from West Africa and put them in a pantropical perspective.

5.5.1. Deceptive pollination in Mediterranean orchids: past, present and future

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The present contribution is an attempt to review the field along an artificial time scale.

Past: this stage includes the recognition and description of the various deception and mimicry types and the shaping of the main hypotheses; most of which are based on field observations. The basic reviews were published from 1970 to 1992. Present: 1. Experimental testing of the role of actual density dependent selection in the evolution of empty flowers including artificial adding of nectar and the study of colour morphs. 2. Fine chemical analysis of floral volatiles of ophrys accompanied with field experiments and eag to identify the attractive compounds and their role in specific attraction in relation to evolutionary pathways of the system. 3. Analysis of floral colours from the viewpoint of bees' colour perception, especially the similarity between models and the mimic, also in relation to odour chemical analysis and odour's pattern activity in the brain of the honey bee. 4. Application of molecular techniques to reconstruct phylogenetic affinities among taxa in relation to speciation, breeding systems, isolating barriers and pollination syndromes. Future: 1. There is an urgent need to study neglected deceptive genera as: *Himantoglossum*, *Cephalanthera*, *Traunsteinera* and *Serapias*. 2. There is a room for more multidisciplinary studies as: combination of morphological analysis, breeding systems, molecular genetics, colour analysis, odour analysis and behavioural experiments e.g. in the study in

closely related species and in hybrids of *Ophrys*, *Orchis* and *Serapias* to elucidate processes of sympatric speciation. 3. Use of optical pattern analysis to compare the similarity between female bees and *Ophrys* in relation to male response. 4. Study of the possible adaptive value of the high variability of colour patterns (especially in *Orchis* s.l.) in relation to bee's perception and the individual plant variability.

5.5.2. Scent variation and its role in hybridisation and speciation of sexually deceptive orchids of the genus *Ophrys*

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Ophrys orchids mimic the sex pheromones of female insects and thereby attract males for pollination. We investigated intra- and interspecific variation of pollinator attracting scent with the aim to see whether speciation is driven by changes in floral odour. In different populations within a species, the pollinator attracting compounds show only minor differences, whereas the differences are greater in compounds that do not have a function in pollinator attraction. This finding suggests a pollinator driven selection on the male attracting scent. This hypothesis is supported by our finding that *Ophrys* species with the same pollinator - independent of their phylogenetic relationship - use almost identical odour bouquets for pollinator attraction. The results of a further investigation, in which we compared the scent of hybrid swarms and sympatrically occurring *Ophrys* taxa of the *O. fusca* group, support the hypothesis that hybridisation can be a first step for speciation in sexually deceptive orchids and that speciation is probably driven by changes in floral odour. Supported by the DFG (AY 12/1-1)

5.5.3. Lying to Pinocchio: deceptive mimicry in orchids pollinated by long-proboscid flies

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Plants that lack floral rewards may nevertheless be attractive to pollinators if their flowers effectively mimic those of co-occurring rewarding species. This form of mimicry (floral Batesian) has evolved in several lineages within the orchid genus *Disa* and is strongly associated with specialized pollination by long-proboscid flies belonging to the families Nemestrinidae and Tabanidae. Mimicry may be especially prevalent in long-proboscid fly pollination systems because scent (a trait difficult to mimic because of its chemical complexity) appears to play little or no role in attraction of these flies. Experiments show that flies generally do not distinguish between these orchids and their models, presumably due to their closely matched spectral reflectance and morphology. The orchids thus achieve high rates of pollination when intermingled with models, and are ecologically dependent on them. Although flies do not discriminate between the orchids and their models, they probe far fewer flowers on the orchids. This behaviour is a response to the lack of nectar and effectively reduces geitonogamous self-pollination.

5.5.4. How pollination webs unravel: landscape-scale variation in pollination in a guild of oil-secreting orchids at the Cape of Africa.

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The diversity and composition of a community of oil-secreting orchid species (Coryciinae) was found to be related to the rate of pollination by oil-collecting bees (*Rediviva* spp.). Species diversity was positively correlated with increasing pollination rate. Species composition followed a predictable sequence. Species with high demographic dependence on pollination occurred only where the pollination rate was high. Low demographic dependence was indicated by a capacity for self-pollination or vegetative reproduction, features lacking in species with high demographic dependence on pollinators. Sharp boundaries in the distribution of oil-collecting bees were detected. These boundaries coincided with soil-type boundaries. Outside the distribution range of oil-collecting bees, populations of some oil-secreting orchid species persisted by forming massive clones, others evolved novel floral features to attract alternative pollinators or facilitate self-pollination.

5.5.5. Pollinator attraction in orchids: from molecules to evolution

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Sexually deceptive orchids mimic mating signals of female insects and attract males which pollinate the flowers in an attempted mating. Pollinator attraction is usually species-specific and mediated by the floral odor. In the Australian *Chiloglottis trapeziformis*, one compound attracts a thynnine wasp, the sole pollinator of this orchid. In other *Chiloglottis* species, similar molecules are the pollinator attracting signals. Small changes in genes encoding odor producing enzymes may thus be responsible for reproductive isolation mediated by species-specific floral odor in this genus. In the European genus *Ophrys*, blends of many biologically active compounds attract the bee-pollinators. In *O. sphegodes* and *O. exaltata*, we found the same active odor compounds, but in significantly different relative amounts. In these species, the different expression of genes encoding odor producing enzymes may thus be the mechanism for species-specific odor signals. In both the Australian and European system, sympatric speciation is imaginable through changes in floral odor, a signal that is linked to reproductive isolation through specific pollinator attraction.

5.5.6. Food is good but sex is better: the evolution of deceptive pollination in the tribe Diurideae (Orchidaceae)

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A molecular phylogeny for genera of the tribe Diurideae was used as a framework for reconstructing character phylogenies for published data on reproductive attributes, including nectar production, mimetic system, breeding system and pollinator families. For some of these, results are equivocal but sexual deception has evolved independently in at least five different lineages, usually from food-mimicking ancestors. The only unequivocal evolutionary transformations from sexual deception are to autogamy and apomixis. Selective pressures that might have driven these trends will be discussed. The hypothesis of co-speciation between orchids and pollinators has been tested and rejected in the sexually deceptive genus *Chiloglottis*. Pollinator switching is the dominant mode of pollinator diversification in this genus and is probably so in other sexually deceptive clades. The genus *Diuris*, which includes both rewarding nectar producing species and deceptive food mimics, offers novel opportunities for future comparative and experimental analyses of the evolution of deceptive pollination systems.

5.5.7. Floral mimicry in Costan Rican Oncidioid orchids

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Oncidioid orchids (subtribe Oncidiinae) demonstrate an extraordinary radiation, comprising over 1700, largely non-rewarding, species in tropical America and have been hypothesised to mimic oil-producing members of Malpighiaceae on the basis of floral morphology. We evaluate the role floral mimicry has played in the radiation of these orchids in Costa Rica by analyzing colour distributions and floral reflectance of sympatric plants from 32 sites, where either an oncidoid orchid, or a yellow-flowered Malpighiaceae species, was central to the site. As bees are the most common pollinators in these communities and almost certainly the pollinators of both Malpighiaceae and oncidoid orchids, we measured floral colour reflectance from 300-700nm to conduct the study. Preliminary analyses of the data indicate that two principal types of orchid mimicry are in operation: (i) oncidoid orchids of the genera *Oncidium* and *Erycina* reflect both yellow and ultra-violet light, a characteristic shared with some yellow-flowered Malpighiaceae; and (ii) other oncidoid orchids tend to converge on the rest of the community without displaying any unique differences.

5.6.1. It is not just a flora anymore

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We live in a time of unprecedented loss of biodiversity and global homogenization. With funding for exploration, description, and a refined understanding of species being a low global priority, traditional approaches to writing floras are no longer adequate. Therefore, we need to find more efficient methods to increase productivity. We must develop an internet-based system to reduce the high level of redundancy in floristic and monographic work, in which descriptions and other detailed (including phylogenetic) information on species, genera, etc., are written into an online database and made available for online and paper-based flora projects, monographs and publication of new taxa. Some data by their nature must be maintained by distributed data centers linked via portals (e.g., collections) while other information should be centralized (e.g., taxon information and nomenclature). Plant information developed in a collaborative online system would allow data on species and higher level groups to appear on Web sites as soon as they are written and edited, and immediately after new information or taxa are published.

5.6.2. Floristics and global information infrastructure

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Stimulated by the establishment of the Global Biodiversity Information Facility (GBIF) in 2001, a large network of distributed biodiversity resources is quickly developing worldwide. In its first phase, GBIF has focused on providing access to metadata, taxonomic names, and to specimen and observation data, by establishing XML-based web services, a collection of protocols and standards used for exchanging data between computer applications. Much of the information associated with floristic work such as keys and descriptions has remained unused by lack of appropriate standards to meaningfully aggregate data from various sources. A key challenge is that providers of this information do not use the same terminology for similar concepts. Emerging solutions include the development of a TDWG standard to deal with descriptive data (SDD). Emerging challenges include integration with existing ontology efforts.

5.6.3. Floristics for the 21st century: Flora of North America as a case study

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Flora of North America (FNA) has the goal of providing authoritative information on the plants of North America north of Mexico to a wide range of users. The project has been guided by "Floristics for the 21st Century," which concluded that computer technology could be an important operational tool and a powerful vehicle for making information available. With 1/3 of the project completed, we find that technology has done both more and less than anticipated. FNA meets two key requirements for analysis of floristic data: comparable descriptions and geographic distribution, which are provided in a relational database format. On the Internet, hypertext and search engines have been more important than relational databases for accessibility of data, however. The online availability of references and herbarium collections have improved the quality of treatments. Review and editing processes have been served better by simple off-the-shelf programs than by computer-assisted collaborative work processes. Perhaps most importantly, the fundamental requirement to complete floras is an expert botanical and editorial workforce, which cannot be replaced by computers.

5.6.4. The Jepson Flora Project: An integrative approach to floristics

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The Jepson Flora Project is a worldwide botanical collaboration dedicated to providing detailed, rigorous, and current information on the California flora through innovative use of electronic and printed media. Rapid documentation of newly discovered, native and naturalized plant taxa in California and advances in understanding of relationships, ecological attributes, distributions,

and endangerment of California plants makes integrative approaches to floristics an urgent need. Such integration depends on using classifications that reflect phylogeny and that represent the full diversity of well-supported, minimal evolutionary lineages. Achieving that goal is challenging for California, where recent diversification of lineages, sometimes accompanied by unequal rates of phenotypic evolution, has resulted in much cryptic diversity and in groups of phenetically similar taxa that are not clades. Floristic integration by the JFP also involves continuing development of on-line resources for uses ranging from plant identification, to horticulture, to flora-wide analyses of ecological and biogeographic questions in a phylogenetic framework.

5.6.5. Bryophyte floras in the digital age: floras for those who cannot afford them

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Africa is a country desperately in need of floras for bryophytes and other lower plants. There are significant difficulties in encouraging and supporting studies in this area, when all the literature and specimens that are needed are outside of Africa, and where only South Africa has a published bryophyte flora (although not yet complete). Bryology is not well-funded, and attempts to get funding have had only limited success. Nevertheless, good progress is being made on a generic flora for bryophytes (approximately 60% completed), but in the longer term the target is Bryologia Africana, a species level flora for tropical Africa. It will take some time to describe almost 5000 taxa, so we intend initially to publish the generic flora, as it is produced, on the web, and then add keys to species and descriptions as they become available, as part of a future Bryologia Africana: a full, free, on-line bryophyte flora for tropical Africa. If we can publish on paper we will, but the most important factor is making the information available as a free resource.

5.6.6. Floristic challenges in Latin America at the beginning of the XXIst century

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Molecular biology, biotechnology and genetic engineering are main scientific areas receiving interest and support from the scientific community and funding institutions. Along with this situation, there has been a significant decline in supporting basic research activities that still deserve attention. This is the case of the floristic work that includes data of plant inventories such as checklists, floras, species distribution pattern data and assessments of plant diversity and endemism in different regions. In the beginning of the twenty first century, many megadiverse countries of the world still lack a catalogue of their flora, and some of them are far away of having one in the near future. In Latin America, many countries have developed important programs to increase the knowledge of their plants, either by their own efforts or with the help of other countries. Each country has designed its own approach for cataloguing its flora, thus, the results and accomplishments obtained are diverse. A summary of the major floristic programs currently being undertaken in Latin America and a discussion regarding their present and future activities is presented.

5.6.7. Flora brasiliensis revisited - a website to improve knowledge of the Brazilian flora

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A century after the completion of Martius' *Flora brasiliensis*, the Brazilian flora is still poorly known. Estimations of the number of angiosperm species there vary from 30,000 to over 60,000. Taxonomic work there is a good example of the "taxonomic impediment" for exploration and management of the earth's biodiversity. Progress on the Brazilian flora continues with too few taxonomists and difficult access to historical specimens, literature, and associated data and imagery. Our project works on creating a database-fed website that will allow the user to quickly find the most relevant data for each taxon. A wide array of specialists will be asked to participate for different angiosperm families. The starting point is the 40-fascicle *Flora brasiliensis* (1840-1906), which includes nearly 25,000 described species and close to 4,000 high quality lithographs. The initial steps include updating the

names in the *Flora*, which is still widely used to identify specimens, and digitizing the illustrations. Other historical works, more recent data, and other illustrations and photographs will complement the historical data on the website.

5.7.1. The streptophyte algae: thirty years after

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In two seminal contributions, Jeremy Pickett-Heaps (1975) and Ken Stewart and Karl Mattox (1975), shifted the prevailing paradigm about the phylogeny and systematics of the green algae, namely that green algal evolution followed a linear sequence of organizational levels starting with flagellates and terminating with differentiated thalli. These authors identified ultrastructural characters (mostly of the flagellate and mitotic/cytokinetic cytoskeleton) which strongly suggested that there exists a motley assemblage of morphologically diverse green algae (which they termed Charophyceae) specifically related to embryophyte plants and from whose ancestors the latter were postulated to have evolved. This proposal has been spectacularly confirmed by molecular phylogeneticists during the 1990s and remains one of the best examples of congruence between morphology and molecules. The exact relationships of the streptophyte algae to each other and the likely steps that led to the evolution of embryophyte plants, however, are still eluding us today. Multi-gene (genome) phylogenies and transcriptome comparisons are helping to unravel these questions.

5.7.2. EST analysis of the scaly green flagellate *Mesostigma viride* (Streptophyta): Implications for the evolution of green plants (Viridiplantae)

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The Viridiplantae are grouped into two monophyletic groups: the Chlorophyta and the Streptophyta. The Streptophyta include all embryophytes and a diverse group of freshwater algae known as Charophyceae. The only scaly flagellate included within the Streptophyta is *Mesostigma viride*. The exact position of *M. viride* within the Streptophyta is still controversial. We present an EST-analysis of *Mesostigma viride* and compare the ESTs of *Mesostigma* with the available plant genomes (*Arabidopsis*, *Oryza*, *Chlamydomonas*) and ESTs from *Physcomitrella*. In general, plastidic and mitochondrial metabolic pathways, proteins of the cytoskeleton and proteins involved in protein folding are better conserved between *Mesostigma* and *Chlamydomonas* than between *Mesostigma* and the embryophytes. In contrast, cytosolic metabolic pathways, and proteins involved in vesicular transport, transcription, regulation, DNA-structure and replication, cell cycle control, and RNA-metabolism are better conserved between *Mesostigma* and the embryophytes than between *Mesostigma* and *Chlamydomonas*.

5.7.3. Charophyte green algae and the origin of embryophytes from the perspective of 2005

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In recent decades application of ultrastructural methods, formal phylogenetic analysis, and most recently molecular systematic data, has provided a clear view of the evolutionary origin of embryophytes (land plants). Multi-gene molecular analyses support the idea that the Charophyta sensu Mattox and Stewart are a fundamental lineage of green algae that also includes embryophytes. The Charales appear to be the sibling taxon to embryophytes, confirming the Streptophyta sensu Jeffrey 1967. The Coleochaetales are sibling to that clade. The highly diverse Zygnematophyceae have been considered problematic because of their distinctive mode of reproduction, but they are clearly placed among the charophytes, and are more closely related to embryophytes than are any algae other than the Charales and Coleochaetales. Also important are structurally simple, basal taxa that represent extremely ancient lineages. As phylogenetic relationships among these organisms becomes more definite, the challenge will be to combine phylogenetic information with knowledge of the biology of the organisms to provide new insights into the origin and evolution of embryophytes.

5.7.4. Morphological variation and genetic diversity in *Chara braunii* (Charales, Charophyceae) in Japan

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Chara braunii (Charales, Charophyceae) is a cosmopolitan species, and occurs in various environments, such as small/shallow ponds, paddy fields, large/deep lakes etc. *C. braunii* exhibits large morphological variation and R. D. Wood (1965) classified this species into seven forms, on the basis of differences in morphology of bract-cell, stipulode, and oospore (zygote). However, no studies on the relationships between morphological variation, habitats and genetic diversity in *C. braunii* have been carried out. The present study was undertaken to evaluate the species concept of *C. braunii* using culture methods and DNA sequence data. We have collected many samples from various localities in Japan including paddy fields and lakes etc. The present *rbcl* gene sequence data resolved robust two clades of *C. braunii*, and samples collected from lakes belong to only one of the two clades. Morphological variation and habitats of *C. braunii* will be discussed in relation to the phylogenetic relationships within the species.

5.8.1. Angiosperm phylogeny - progress and prospects

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The publication of the APG system in 1998 summarized a decade of unparalleled progress in understanding angiosperm phylogeny. The robustness of the results was demonstrated by the few changes that were made in the APGII update in 2003. Unresolved issues include some major interrelationships at and above the ordinal level and many unclear interrelationships within orders and families, where extensive work remains to be done. Furthermore, the APG principles of classification may be debated. This presentation discusses what could be expected from a future APGIII update. The expanding area of dating angiosperm phylogenies is also addressed. The identification of all angiosperm 100-my-clades is an important future goal. Many orders and even some families of the APG classification comprise several such 100-my-clades and some 100-my-clades comprise several APG orders. Orders and families of the APG classification are essentially arbitrarily circumscribed (albeit monophyletic) and not of comparable age. The 100-my-clades are of similar age and hence more useful entities in research on angiosperm evolution.

5.8.2. Life under stress: order Caryophyllales

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The broadly defined order Caryophyllales (sensu APGII) includes families adapted to extreme environments, from dry, salty conditions to oligotrophic soils. Since publication of the first angiosperm DNA analysis, composition of the order has relatively remained unchanged. Nevertheless, two main areas of research need to be addressed: relative position of the order within eudicots and relationships among families. Although Dilleniaceae are consistently placed as sister group, support for this relationship remains weak. In addition, the position of Caryophyllales/Dilleniaceae within eudicots remains unresolved. Analyses of individual genes consistently recover two main lineages, but many relationships within these two groups are still difficult to assess. Non-monophyly of some widely accepted families (e.g. Cactaceae, Portulacaceae, Phytolacaceae) makes complex, or at least controversial, attempts to reclassify the group. Synapomorphies defining the caryophyllids will be discussed.

5.8.3. Rosid angiosperms: circumscription, issues, and systematic advances

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The rosid lineage comprises nearly one-third of all angiosperm species and is classified into 140 families, most of which are placed in 15 orders. The position of Vitaceae relative to other

rosids and to other tricolpates is still uncertain. Two major lineages, fabids and malvids, are recognized within rosids. Three other orders, most notably Myrtales, are still unplaced within rosids. Familial relationships within many orders are now clarified, although some, especially Malpighiales, are not due to apparent rapid rates of diversification early in their evolution. Rosids are relatively heterogeneous with respect to diagnosable morphological features as compared to asterids. Major breakthroughs in delimiting rosid subclades include those defined by the propensity to fix nitrogen or by the production of glucosinolates with specialized myrosinases. Morphological and anatomical characters define many orders, but few more inclusive groups within the rosids. Rosids are the focus of several large scale studies incorporating fossils into molecular phylogenies to understand both temporal and spatial diversification of the group.

5.8.4. Phylogenetic relationships and evolution in basal angiosperms

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Recent collaborative, multigene analyses have identified the basalmost nodes of angiosperm phylogeny. Amborellaceae are generally sister to all other angiosperms, followed by Nymphaeaceae and Austrobaileyales. Other lines of evidence further support these relationships. For example, a deletion in exon 5 of homologues of the floral MADS-box gene PISTILLATA unites all angiosperms except Amborella and Nymphaeaceae. Despite considerable progress in angiosperm phylogenetics, critical deep relationships remain unclear. "Above" the three basal branches, monocots, Ceratophyllaceae, Chloranthaceae, the magnoliid clade, and eudicots essentially form a polytomy. Genomic studies have provided key insights into the evolution of basal angiosperms. For example, basal angiosperms are ancient polyploids. Furthermore, they have a complex array of floral developmental genes, comparable to those in core eudicot models such as Arabidopsis. However, expression patterns of floral MADS-box genes are generally broader in basal angiosperms than in core eudicot models, perhaps accounting for differences in floral organization.

5.8.5. Higher-level relationships in the monocotyledons: multi-gene analyses

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Phylogenetics relationships among monocots have over the past ten years been the focus of a number of large-scale phylogenetic studies, which have to a large degree resolved most of the major questions. Based on analyses of seven genes representing all three genomes, *Acorus* (Acorales) is sister to the rest, followed at the next several nodes by Alismatales (including Araceae and Tofieldiaceae), Petrosaviales, Dioscoreales/Pandanales, and finally Asparagales sister to the commelinids. All of these receive at least moderate bootstrap support, but most are greater than 90%. Within commelinids, Commelinales are well supported as sister to Zingiberales, but it is not yet clear how this pair or orders is related to Arecales, Dasypogonaceae and Poales. This general topology is consistent with an aquatic or semi-aquatic origin of monocots; leaves with reticulate venation are not the ancestral condition, in contradiction to results of some morphological cladistic analyses. Studies of molecular clocks have indicated that the crown node of monocots is 127-134 million years old, and most orders and some families are older than 100 million years.

5.8.6. Asterid phylogeny - updated and dated

J. Kärehed, B. Bremer;

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The asterids, representing ca. 1/3 of all angiosperms or over 80 000 species, was one of the first major clades to be molecularly investigated. Less than fifteen years later the general picture is the same but many more taxa have been investigated and several molecular markers have been analysed with similar results. Both APG classification systems (APG and APG II) accept ten orders (Cornales, Ericales, Garryales, Gentianales, Lamiales, Solanales, Aquifoliales, Apiales, Asterales, and Dipsacales) and more than 100 families (107 and 101, respectively). All ten orders have stem nodes dated back to early Cretaceous (128-106 my) and crown nodes dated from early to late Cretaceous (114-78 my). The orders are well supported but relationships between several orders

are, despite intense trials, not resolved. Focus of asterid researchers today is to resolve the relationships within the families, particularly of the species rich as for example Asteraceae and Rubiaceae.

5.9.1. Phylogeny of the Ericales

A. A. Anderberg¹, J. Schönenberger²;

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Analyses of DNA sequence data have shown that Ericales is monophyletic and comprised of 23 families from three subclasses (Asteridae, Dilleniidae, Rosidae), and that they are sister to the Euasterids. Relationships among families and several family circumscriptions have been amended. Balsaminaceae, Tetrameristaceae + Pellicieriaceae, and Marcgraviaceae are in a clade that is sister to the rest. The largest group is formed by (1) Clethraceae, Cyrillaceae, and Ericaceae as sister to Actinidiaceae, Sarraceniaceae, Roridulaceae, (2) Diapensiaceae and Styracaceae sister to Symplocaceae, and (3) Theaceae s.str. Outside of this group, (4) Sapotaceae is sister to Ebenaceae and the primuloid families, and (5) Theaceae-Ternstroemioidae group with Pentaphylacaceae and Sladeniaceae. Unresolved outside of the major clade are (6) Lecythidaceae, and (7) the Fouquieriaceae as sister to Polemoniaceae. The results are important for studies of character evolution, and although the Ericales lack apparent morphological synapomorphies, states such as sympetaly, unitegmy, and cellular endosperm are shown to be characteristic of certain clades.

5.9.2. The phylogenetic significance of wood anatomy within Ericales

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Ericales sensu APG show a large wood anatomical variation providing significant information for systematic relationships within the order. The so-called primitive Baileyan features (e.g. mainly solitary vessels with scalariform perforations, opposite to scalariform vessel pitting, distinctly bordered fibre pits, scarce apotracheal parenchyma, heterocellular rays) are common in ericoids, Pentaphylacaceae, Symplocaceae, Styracaceae and Theaceae. On the other hand, balsaminoids, Ebenaceae, Lecythidaceae, primuloids and Sapotaceae are characterised by radial vessel multiples, simple perforations, alternate vessel pitting, and fibres with simple pits. Within the order, there is good support for the current delimitation of various subclades (e.g. balsaminoids, ericoids and primuloids). In addition, the wood structure provides evidence for a close relationship between Theaceae and Symplocaceae on the one hand, and between Ebenaceae, Lecythidaceae and Sapotaceae on the other. Below family level, wood anatomical data show systematic significance in families such as Ericaceae, Lecythidaceae and Sapotaceae.

5.9.3. Phylogeny and biogeographical comparison of Diapensiaceae, Styracaceae, and Symplocaceae.

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Recent molecular phylogenetic analyses of order Ericales have demonstrated strong support for a clade comprising the families Diapensiaceae and Styracaceae, with Symplocaceae as sister-group. I review current knowledge on the internal topology of these families based on morphological, molecular, and combined results, summarize putative synapomorphies for the major subclades, and present proposals for revised infrafamilial classification. I also compare biogeographical patterns of these groups based on tree topologies and current understanding of fossil histories. Styracaceae and Symplocaceae share remarkably similar distributions, occurring in tropical and warm north-temperate regions of the Americas and eastern Asia, with one Mediterranean species in Styracaceae. Event-based biogeographical analyses with molecular-based topologies support an eastern Asian origin and subsequent Tertiary expansion into South America (probably via dispersal from tropical North America) in each family. A study in progress of abundant endocarp fossils of Symplocaceae will test biogeographical interpretations based on molecular phylogenetics.

5.9.4. Bayesian phylogenetic analysis of multiple chloroplast markers contributes new knowledge about Sapotoideae (Sapotaceae) phylogeny

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Swedish Museum of Natural History, Stockholm, Sweden.

The pantropical clade Sapotoideae contains more than 500 species of trees and shrubs, whose phylogenetic relationships are poorly understood. We present a study based on a chloroplast DNA data set of more than 5 kb for 66 Sapotoideae species. Our results show that a couple of taxa previously placed in this group do not belong here. Eberhardtia aurata is the sister of the two major Sapotaceae clades; Sapotoideae and Chrysophylloideae, and Neohemsleya usambarensis belongs in Chrysophylloideae. Sapotoideae consists of the well supported Sideroxyloae and a grade of taxa placed in Glueminae and Isonandreae. Short internal branches within this grade make it difficult to resolve with good support. Within the grade a strongly supported clade with more complicated floral structure is nested. This clade more or less corresponds to Mimusopinae and Manilkarinae. The effect of model choice on the resolution of short branches in Bayesian phylogenetic analysis is discussed, as well as character evolution of floral traits and possible implications for the classification of the group.

5.9.5. Systematics of Sapotaceae and a new subfamily classification

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Parsimony analyses of Sapotaceae, based on morphological and molecular data, confirm that the family circumscribes three main evolutionary lineages corresponding to the tribes (1) Isonandreae-Mimusopeae-Sideroxyloae, (2) Chrysophylleae-Omphalocarpeae, and (3) the genus Sarcosperma, respectively. The Sideroxyloae are monophyletic, Isonandreae are polyphyletic, and the subtribe Mimusopinae have evolved within the Mimusopeae-Manilkarinae. The large tribe Chrysophylleae are monophyletic only if the taxa from tribe Omphalocarpeae are included. This clade includes the large genera Chrysophyllum and Pouteria, neither being monophyletic in their current circumscription. An amended subfamily classification has been proposed that corresponds to the monophyletic groups Sarcospermatoideae (Sarcosperma), Sapotoideae (Isonandreae-Mimusopeae-Sideroxyloae), and Chrysophylloideae (Chrysophylleae-Omphalocarpeae), where Sapotoideae circumscribes the tribes Sapoteae and Sideroxyloae. Relationships in and among the latter tribes are yet unclear.

5.9.6. Evolution of new world Lecythidaceae

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Molecular data from the *trnL-trnF* and *ndhF* genes suggest that the generic limits of *Corythophora*, *Couratari*, *Couroupita*, *Grias*, and *Gustavia* represent distinct clades as currently defined with *Grias* and *Gustavia* basal to the remaining Lecythidoideae. *Cariniana*, on the other hand, comprises two groups: one with actinomorphic, fleshy flowers and the other with zygomorphic non-fleshy flowers. Poor resolution among the remaining zygomorphic-flowered species does not clarify the relationships of *Bertholletia excelsa* (the Brazil nut), *Corythophora*, *Eschweilera*, and *Lecythis*. Most of the species of *Eschweilera* form one large clade characterized by seeds with a lateral aril. The remaining species of *Eschweilera* are scattered among species of *Lecythis*. Species of *Lecythis* are found in at least four places in the *trnL-trnF* and *ndhF* trees and species that morphologically have nothing in common appear in the same clade. The currently recognized sections of *Lecythis* (*corrugata*, *pissonis*, *poiteaui*, and *Lecythis*) are only supported for section *corrugata* in the *ndhF* tree.

5.9.7. Morphological diversity and biogeography of Ericales

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The newly circumscribed Ericales encompass a range of morphological diversity that rivals that of most other eudicot orders. Many taxa possess highly specialized features such as the nectariferous bracts in Marcgraviaceae and the tendency to accumulate metals such as aluminum (Symplocaceae). Members

of this clade vary in their geographic distribution from highly restrictive (Pellicieriaceae) to pantropical (Sapotaceae) or cosmopolitan (Ericaceae). The core Ericaceae (Actinidiaceae, Roridulaceae, Sarraceniaceae; Clethraceae, Cyrillaceae, and Ericaceae) frequently occur on acidic or nutrient poor soils. Relationships among core Ericaceae are analyzed via parsimony and maximum likelihood using molecular data. Morphological features mapped onto the resulting cladogram indicate that carnivory evolved once and was lost in the ancestor to Actinidiaceae. Analysis of the geographic distribution of representatives within core Ericaceae indicates that North America may have been an important area of diversification during the mid- to late-Cretaceous.

5.10.1. History of botany in Austria

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Already in the late 16th century emperors employed botanists like Matthioli & Clusius, who produced the first flora of higher plants & the first fungus flora. Foundation of the botanical gardens of the University of Vienna & Schönbrunn 250 years ago. Jacquin introduced the binary nomenclature of Linnaeus; Endlicher & Wettstein developed new systems of classification. Expeditions to Central and South America (Jacquin, Boos, Pohl, Schott, Mikan, Haenke), South & West Africa (Boos, Welwitsch), Australia, Southwest Asia & N. Africa (Sieber, Kotschy), Hawaii (Wawra, Rick) or China (Rick, Handel-Mazzetti) brought rich materials for the Imperial collections. Floras of Austria & its provinces & of the Balcan Peninsula were written by authors like Jacquin, Host, Schultes, Wulfen, Neilreich, Halacsy, Hayek & others. The most outstanding Flora of recent times is Rechinger's Flora Iranica. Cryptogamic botany: From Wulfen to Zahlbruckner, Petrak & Geitler. For palaeobotany Unger & v. Ettingshausen, for plant physiology pioneers like Ingen-Housz & famous representatives like Unger, Wiesner & Molisch are to be mentioned; Kerner is one of the founders of plant ecology.

5.10.2. Austrian botanical explorations in Iran

H. U. Riedl, H. U. Riedl;

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Starting from the extensive travels in northern and southern Iran of Th. Kotschy in 1842 to the voluminous "Flora Iranica" edited and partly composed by the late K.-H. Rechinger, who also excelled as a collector, since 1963 and now almost completed there was a permanent interest for the flora of this country in Austria. While Th. Kotschy, O. Stapf, K.-H. Rechinger, E. Gauba and A. Gilli were trained as botanists important contributions were also made by physicians to the court of the Shah like J. E. Pollak together with Th. Pichler and J.A. Knapp in the 19th, geographers like A. Gabriel and H. Bobek or zoologists like A. Ruttner-Kolisko, F. Kasy and J. Eiselt in the 20th century. Their collections either preserved at the Vienna Natural History Museum or in the herbarium of Vienna University were all identified and/or revised for "Flora Iranica" and include a great number of nomenclatural types.

5.10.3. K. K. Münch-Bellinghausen's herbarium in the Moravian museum and its evaluation

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K. K. Münch-Bellinghausen (*10.6.1752, +11.11.1838) created a herbarium collection of more than 10 000 specimens in the years 1800-1838. Its major part was acquired by purchase from various botanists. Bold letters in the list below indicate collectors their specimens were up to this time elaborated in special studies. They will be object of digitalisation in the next time. Balbis, Benesch, Besser, Blandow, Bonjard, Bonjean, Buek, Burkhardt, Carl, Crome, Dierbach, Dietrich, Duval, Eisenstein, Flotow, Freyer, Gaertner (+ Meyer, Scherbius), Haller fil., Heckel, Herbich, Hess, Hinterhuber, Hoppe, Host, Hübner, Huguenin, Jahn, Kiteibel, Klotzsch, Kosteletzky, Kotschy, Láng, Lejeune, Mann, Masner, Mertens, Münch, Noe, Nolte, Opiz, Panzer, Peterman, Portenschlag, Presl, Reichenbach, Rohde, Rochel, Rudolphi, Ruprecht, Rümmelein, Sadler, Salzmann, Sauter, Schleicher, Schmalz, Schultes, Seidl, Sieber, Thomas, Traufelner, Traunsteiner, Vanderschoff, Wahlenberg, Waldstein, Weihe, Welden, Welwitsch, Wierzbizki, Zawadzki, Ziz.

5.10.4. Richard Wettstein von Westersheimb and his father in law Anton Kerner von Marilaun

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The research of the nature with new technologies and a big number of students at the University of Vienna at the end of the 19th century needed new structures within the universities institutions, and an appropriate arrangement with the academic learned persons. Two well known botanists of this area (the end of the Hapsburg monarchy) were Anton Kerner von Marilaun and his son in law Richard Wettstein von Westersheimb; both directors of the botanical garden of Vienna. Especially the biography of Richard Wettstein sounds absolutely phantastic. Born in Vienna in 1863, he married in the age of 27 the daughter of Anton Kerner, Adele, and, a little bit later, became director of the botanical garden in Prague. After Anton Kerner's death, he also became director of the botanical garden in Vienna, Rennweg 14. In this function he rebuilt the building of the botanical institute in Vienna in a very short time. In 1905 he organised the Second International Botanical Congress in Vienna, exactly 100 years ago.

5.10.5. Discovery of photosynthesis. Jan Ingenhousz - his experiments and life

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Natural History Museum, Vienna, Austria.

The effect of animal respiration spoiling the air and its reversal by plants was discovered by Joseph Priestly (1772). Unfortunately he did not perceive the light dependency. So he was confused by diverging results of several experiments, finally became doubtful and cannot be regarded as the discoverer of photosynthesis. Inspired by Priestley's experiments, the Dutch physician and expert on small pox inoculation at the imperial court of Maria Theresia, Jan Ingenhousz (born 1730 in Breda, died 1799 near London) found a method to observe the very moment when the restoration of bad air would begin: Putting the plants under water to see the "pure air" or "dephlogisticated air" (today we call it oxygen) oozing out as bubbles only in light, mostly from the lower side of fresh leaves and "not owing to the warmth of the sun* but* to the light". Though - after 500 well planned experiments in summer 1779 and publishing the results end of 1779 - Ingenhousz gave full credit to the pioneering role of J. Priestley. The quarrelsome English Chemist and priest would not put up with having missed the factor and attacked Ingenhousz. Benjamin Franklin, admiring both of them, tried to mediate. Ingenhousz who was highly respected at the Viennese Court (1768-1779) had personal contacts with N. v. Jacquin (his brother-in-law), with the young W.A. Mozart, with Mesmer (the controversial doctor curing with the help of "animal magnetism", glass organ music and hypnosis) and with A. Fontana in Florence (to whom he owed the method of analyzing the purity of "dephlogisticated air", based on oxidation of the insoluble NO-gas to the water soluble NO₂). The author has repeated some of Ingenhousz' experiments and traces back his life lines, his amiable character, many-sidedness and scientific ingenuity.

5.10.6. The golden century of austrian botany

H. Lack;

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The golden century of Austrian botany is an appropriate term for the years 1750 to 1850. During this period the first chair of botany and the first botanic garden were founded in what is now Austria - at Vienna University. A botanic garden was added to the imperial summer residence of Schönbrunn near Vienna, the k. k. botanisches Hofkabinett, part of the imperial collections, but effectively a public herbarium, was created, and very substantial collections of botanical books and illustrations were added to the imperial library in Vienna. The key figures were Nicolaus Joseph Freiherr von Jacquin, his son Joseph Franz and Stephan Ladislaus Endlicher, the second, third and fourth professor of botany, all focused on plant diversity then only very incompletely known. Emperor Franz Stephan of Lorraine and Franz I, Emperor of Austria greatly encouraged the Jacquins and their circle. During this period the basic dichotomy of botany in Vienna still in existence developed - the Natural History Museum and the Botanical Institute being the largest depositories of botanical collections and centres of biodiversity research in Austria.

5.10.7. The scientific significance of Ferdinand Bauer's Pacific drawings

D. J. Mabberly;

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Since the pronouncements of Goethe, the work of the Austrian painter, Ferdinand Bauer (1760-1826), has been considered some of the finest of biological illustration, as it successfully combines unswerving scientific accuracy with aesthetic sensitivity. His watercolour drawings, based on field sketches made on Mathew Flinders's Investigator voyage (1801-3) and subsequently on Norfolk Island and in New South Wales (to 1805), are widely credited with being the finest ever based on specimens in the field anywhere. Bauer's career is briefly outlined, particular note being made of the influences moulding his technique. These included his key initiation in the use and elaboration of colour-coding methods and his working under harsh conditions on Sibthorp's 1786 expedition to the eastern Mediterranean, leading to the monumental *Flora graeca*. The significance of Bauer's meticulous dating and localizing of his field drawings is stressed. The use of his different colour-codes is explored, particularly in his Pacific work, and it is shown that one of his codes was used in the tropics even before he left Europe for Australia. The deciphering of his most elaborate code, using living plants in Western Australia is explained. Its importance today in the 'revival' of extinct plants from Norfolk Island is examined.

5.11.1. What we should (but don't) know about plant clonality

J. F. Stuefer;

Radboud University Nijmegen, Nijmegen, The Netherlands.

Plant clonality and its direct ecological, physiological and evolutionary implication has been a major focus of attention and research activities over the past three decades. Due to this effort we have a reasonably good understanding of characteristic clonal plant features such as resource sharing, ramet dynamics, and the functional relationship between sexual reproduction and vegetative growth in a number of clonal plant species. Nevertheless, the studies which have provided this insight have also generated a considerable number of new and exciting questions, which relate - among others - to ramet communication via non-resource sharing, biotic interactions with herbivores and pathogens, likely effects of clonality on mating system evolution, somatic mutations, intra-plant genetic diversity and the importance of hierarchical selection, the evolutionary background of clonality and phylogenetic correlates between clonality and other life history traits. This talk aims at a critical analysis of the current state of the art with respect to clonal plant research and at the identification of future scientific challenges and possible research directions.

5.11.2. Solving a problem by falling apart: notes on the evolutionary origin of clonality in plants

B. Oborny;

Loránd Eötvös University (ELTE), Budapest, Hungary.

Clonality is a convergent trait in the plant kingdom. Clonal plants share the feature that the genetic individual (genet) is subdivided into multiple physiological individuals (ramets) that are autonomous to some extent. First I discuss the relationship between this subdivision and the basic, modular organisation of the plant body. Then I consider two specific consequences of modularity: 1. disposability of parts of the body, and 2. distributed control of the developmental process. Clonality opens special ways in adaptation to spatial heterogeneity. Individual ramets can parallelly interact with the environment, sampling from the habitat at several locations. Due to functional redundancy, the same genotype can be expressed in various phenotypes, and tested against various environments. Furthermore, different ramets can cooperate by exchanging resources and information. I review the potential selective forces towards and away from clonality, and outline some basic strategies in clonal growth.

5.11.3. Resource sharing in *Aegopodium podagraria*-A study at the genet level

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Plant Ecology and Systematics, Lund, Sweden.

Our aim was to investigate if there are local adaptations in how resources are shared in forest and weedy populations of *Aegopodium podagraria*, and if this is affected by the potential

differences in immigration history. We also compared populations from southern and central Sweden to see how the proximity to the northern distribution limit affected resource sharing. We subjected one ramet of each plant to shadow treatment, shutting out 95% of the available light, while the other had regular day light. The rhizome connection between ramets was severed (no sharing possible) or left intact (sharing possible). The experiment was carried out using ramet pairs, planted in separate pots. We used 5 presumptively different genets (collected 10 m apart) from 4 populations, one forest (heterogeneous, low light levels) and one weedy (relatively homogeneous, higher light levels) population each from south and central Sweden. Ramet morphology and biomass was compared between treatments. I will discuss the results from the experiment in terms of adaptation between populations from habitats with contrasting light climates.

5.11.4. Genetic differentiation in life-history traits between populations of *Mimulus guttatus* with annual and perennial life-cycles

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Institute for Biochemistry and Biology, Potsdam, Germany.

The relative allocation to sexual and vegetative reproduction is among the most important life-history characteristics of clonal plants. In a greenhouse experiment, we tested for genetic differentiation in life-history traits between plants from permanently wet and temporarily wet populations of *Mimulus guttatus*. In the former plants have a perennial life-cycle with efficient vegetative reproduction, while in the latter they have an annual life-cycle. Plants grown from seed from temporarily wet populations produced more flowers, while the ones from permanently wet populations produced more and longer stolons, indicating that the differences in the field represent adaptations rather than sheer plastic responses. Moreover, plants originating from temporarily wet populations produced smaller flowers with shorter anther-stigma separation which is usually associated with higher selfing rates. Our study demonstrates a genetic trade-off between sexual and vegetative reproduction in *M. guttatus*, and strong adaptive genetic differentiation in important life-history traits between temporarily and permanently wet populations of *M. guttatus*.

5.11.5. Flooding gradients: a micro-evolutionary arena shaping trait variation in a clonal herb

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Flooding gradients along rivers are characterized by predictable differences in environmental characteristics, ranging from high levels of disturbance at lower elevations (i.e. close to the water) to high levels of competition at higher elevations. We tested the hypothesis that flooding gradients act as strong selection agents on within-species variation. This implies that genotypes of generalist species should show significant trait differences along flooding-competition gradients (i.e. local adaptation). This prediction was tested by carrying out a common garden experiment with more than 400 genotypes of the clonal herb *Trifolium repens*, collected along several flooding gradients. The results revealed distinctive micro-site-specific differentiation of developmental, architectural and life-history traits within populations. However, the pattern of trait differentiation was not consistent across populations. Selection analyses revealed complex trait interactions in determining plant performance and fitness. This suggests a high potential for natural selection to shape plant traits and population structure along flooding gradients.

5.11.6. Clonal traits are related to life conditions in an Alpine massif (Vanoise)

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¹Cemagref, St Martin d'Hères, France, ²CNRS, Lyon, France.

This study aims to link clonal traits and local-scale environmental variables in 169 herbaceous species plots distributed in the Vanoise Massif (inner part of the French Alps). The clonal traits considered are: duration of clonal integration, length of spacers, and spacer production rate, using the database Clopla1 (Klimeš & al. 1997). We find a significant correlation between the patterns of those functional traits, and environmental variables. Altitude, position in the mountainside, slope, and concave meso-topography are the main structuring factors of these clonal traits. Vegetative growth ability (frequency of multiplication, duration of ramet

integration) tends to increase with increasing climatic stress, although it is less significant for spacer length. This work aims to link, at large scale, clonal structure and environmental factors with harsh and contrasted conditions of stress, and disturbance. The adaptive value of those traits is discussed in the theoretical framework relating environmental restraints and clonality.

5.12.1. Thioredoxin: New functions and applications

B. B. Buchanan;

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Thioredoxins (Trxs) are small proteins that occur in all types of organisms. Nineteen different Trx isoforms grouped in 6 subfamilies and distributed throughout the cell have been identified in the *Arabidopsis thaliana* genome. Trxs contain a conserved redox active disulfide, WC[G/P]PC, that is reduced either (1) photosynthetically by ferredoxin via ferredoxin-thioredoxin reductase in chloroplasts, or (2) enzymatically by NADPH via NADP-thioredoxin reductase in other organelles. Reduced Trxs function as hydrogen donors for the reduction of substrates or, more broadly, the regulation of enzymes. For the first 25 years following its identification in chloroplasts, Trxs were linked to a limited number of proteins. The advent of proteomics has led to the identification of more than 200 potential and confirmed Trx-linked proteins functional in a wide range of processes. The work has uncovered new types of regulation and modes of communication between organelles. Trx appears to regulate processes functional at virtually every stage of plant development. In concert with these advances, new applications in medicine and technology have been described.

5.12.2. Thioredoxin network in the plant cells: what we revealed using thioredoxin affinity chromatography

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Thioredoxin is a small ubiquitous protein, which mediates the dithiol-disulfide exchange reaction with the specific target protein. To list up the possible target proteins of thioredoxin and the related proteins in the cells or certain organelles, thioredoxin affinity chromatography using the immobilized thioredoxin mutants, in which second cysteine is substituted with serine, (Motohashi, K. et al., *Proc. Natl. Acad. Sci. USA*, (2001) 98, 11224-11229) is a useful method. For the past few years, many novel candidate proteins were identified and reported from various organelles and organisms by the proteomics approaches including this method. Information provided by these studies is useful to reveal the complete redox networks around thioredoxins. We will report the recent progress on the analysis of the novel target proteins and will discuss on the merits and demerits of the comprehensive survey methods.

5.12.3. Redox-regulation of carbon storage in response to external signals

P. Geigenberger;

Max Planck Institute of Molecular Plant Physiology, Golm, Germany.

It is discussed how flux to carbon storage is regulated in plants in response to physiological and environmental inputs. Starch is the major storage carbohydrate in plants and ADP-glucose pyrophosphorylase the rate-limiting step of its synthesis. It has recently been shown in potato tubers that this enzyme is subject to a redox-dependent post-translational regulation, leading to a stimulation of starch synthesis in response to the sucrose supply. An analogous mechanism is also operating in photosynthesising leaves, where AGPase redox-activation is independently modulated by light and sugars. Currently, the genetic resources of *Arabidopsis* are being used to investigate the components of the signalling pathways leading to redox-activation of AGPase in response to these inputs. It will be discussed how the sugar signal is transferred to the chloroplast and by which mechanism it interferes with the ferredoxin/thioredoxin system that is involved in the light-dependent redox-activation of the enzyme. Evidence will be presented that a similar mechanism is also regulating the flow of carbon into other pathways such as storage and membrane lipid synthesis.

5.12.4. Strategies to maintain redox homeostasis under changing conditions

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Universität Osnabrück, FB 5, Pflanzenphysiologie, Osnabrück, Germany.

Plants operate well between the extreme situations of over-oxidation and over-reduction caused by the simultaneous presence of oxygen due to oxygen evolution during oxygenic photosynthesis and strong reductants generated in the photosynthetic electron transport chain. Apart from the need for homeostasis of the redox systems, there is obviously need for redox signals that induce the adaptation of metabolism to sustained changes, i.e. during development and upon stress situations. In this presentation, we wish to put forward the hypothesis that a large number of factors is able to induce acclimation and adaptation reactions well before such damage becomes apparent, while the major redox pools are kept constant over a wide range of conditions. Apparently, the responses are broad, rather smooth and depend upon metabolic situation and developmental stage of the cell. We assume that a prolonged period of over-reduction is sensed by the plant already before major imbalances of the redox components and, moreover, long before any oxidative damage becomes apparent. When oxidative stress finally takes over, all cell functions will rapidly and irreversibly break down.

5.12.5. 2-Cys peroxiredoxins as multi-level redox regulated chloroplast antioxidants

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In the network of anthero- and retrograde signals controlling expression, biosynthesis and activity of the chloroplast antioxidant system and photosynthesis 2-Cys Prx are key players. In chloroplasts they reduce H₂O₂ and alkyl hydroperoxides by an intermolecular thiol-disulfide reaction mechanism. High reduction as well as overoxidation of the active site lead to decamerization of the enzyme. Following the conformational change, the enzyme attaches to the thylakoid membrane and modulates photosynthetic activity. The electron pressure on ferredoxin controls gene expression. Signals correlating with a higher acceptor availability of photosystem I decrease the promoter activity via kinase-mediated signalling cascades. Transmission of the redox signal depends on the availability of abscisic acid, whose biosynthesis is affected by violaxanthin cycle activity and the availability of reduced ascorbate in the thylakoid lumen. Redox regulation of gene expression, peroxidase activity and control of photosynthesis define a regulatory circuitry in which the nuclear encoded chloroplast enzyme is a sensor, a regulator and the target of redox regulation.

5.12.6. Is the salicylate signalling pathway a more general redox signalling pathway? Lessons from excess light-stressed *Arabidopsis*.

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The salicylic acid (SA) signalling pathway is well established as a route for the induction of genes that contribute to resistance to a range of biotrophic pathogens. Our recent work indicates that NPR1, a key redox-sensitive regulator of the salicylic acid (SA) signalling pathway, also controls the local and systemic expression of excess light (EL) stress-responsive genes. The functioning of NPR1 may be controlled in part by cellular redox changes brought about by the thiol antioxidant glutathione. In addition to a requirement for NPR1, expression of some EL-responsive genes also requires the action of TGA class basic leucine zipper (bZIP) transcription factors (TFs), which have been shown to form a complex with reduced NPR1 in the nucleus. Depending on the redox state in the cytosol and the nucleus of cells in tissues of stressed leaves, it is proposed that NPR1 may interact with different TGA bZIP TF partners. This would effect changes in the expression of panels of stress-responsive genes, which in turn, would influence physiological responses to different environmental challenges.

5.12.7. Chloroplast redox control of nuclear gene expression

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The light reaction of photosynthesis involves photosystem II (PSII) and photosystem I (PSI) which are connected electrochemically in series. Any imbalance in excitation distribution therefore results in less efficient photosynthesis as observed e.g. under natural light quality gradients. To counterbalance these effects photosynthetic organisms developed acclimation mechanisms which redirect the light energy. As a typical long-term response a photosystem stoichiometry adjustment occurs. This requires a controlled change in the expression of genes which encode the constituents of the photosynthetic apparatus. This control is exerted by redox signals from photosynthetic electron transport which govern the expression of plastid encoded PS core proteins. However, all peripheral PS proteins are encoded in the nucleus and their expression has to be adjusted to respective changes of chloroplast genes. Experiments with transgenic tobacco seedlings and transcript profiles from *Arabidopsis* indicate that the expression of several nuclear genes are controlled by redox signals from photosynthesis representing a new type of the so-called plastid signal.

5.13.1. The novel weapons hypothesis: the role of allelopathy, resistance, and evolution in plant invasions.

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When introduced to new habitats by humans, some plant species undergo astounding increases in dominance. Some invaders may transmogrify because they possess novel biochemical weapons that function as powerful allelopathic agents or as mediators of new plant-soil microbial interactions - the Novel Weapons Hypothesis. My colleagues and I have found that allelopathy appears to be an important mechanism by which *Centaurea maculosa* suppresses native species, and that North American species are more susceptible than related European species. Surviving individuals from North American communities have higher tolerances to the Eurasian invader than individuals from communities that did not experience invasion. Some native species grown from the seed of individuals that survived *Centaurea* invasion are more resistant to the competitive effects of *Centaurea*, root exudates from *Centaurea*, and to a chemical specific to *Centaurea*, (\pm)-catechin. Such effects have implications for plant invasions and the organization of plant communities. Evolved tolerance may ultimately contribute to coexistence among natives and invaders.

5.13.2. Plant root exudates: a novel source of interesting allelochemicals

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Plant root exudates are novel sources of allelochemicals. Using a modified capillary mat system, we collected quantities of root exudates and chemically characterized their constituents. *Sorghum* and *Festuca* spp. produce quantities of bioherbicidal constituents in their root exudates. Sorghums release sorgoleone, a long chain hydroquinone, from living root hair cells. Its MOA in plant growth inhibition includes inhibition of electron flow in photosynthesis and respiration and inhibition of plastoquinone biosynthesis. We have recently characterized a key gene involved in sorgoleone biosynthesis, the SOR 1 gene, a novel desaturase gene. *Festuca* species produce simple novel organic structures with an MOA that involves the inhibition of root cell division or mitosis. Fescue exudates are contained in osmiophilic inclusions in living fibrous root cells. Newer cultivars of fine fescues representing *Festuca rubra* and other *Festuca* spp. provide nearly complete weed suppression of major turf weed species. Further selection and modification of fine fescue weed suppressive potential is underway using both traditional and molecular approaches.

5.13.3. Retarded reforestation: A consequence of the allelopathic potential of invasive Umbelliferae?

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During the 20th century in parts of Europe socio-economic trends caused large-scale land-use changes. Especially the forest belt of Central Europe was abandoned and consequently subjected to secondary succession. A site in Slovenia with extremely invasive and competitive umbellifers, *Laserpitium siler* and *Grafia golaka* - tall herbs are very common in those post-grassland stages -, was chosen to test the hypothesis that umbellifers do not promote reforestation, but, due to the potential allelopathic effect, slow down the succession by self-promotion. Bioassays showed significant inhibition of germination and early ontogenetic development of *Linum perenne*, *Lepidium sativum* and *Festuca rubra* seeds, when treated with extracts or plant organs of umbellifers. Vegetation history of the site was reconstructed with aerial photographs for the last 50 years and a progression of *Pinus sylvestris*, uncommon in montane karst area, was perceived. Germination tests demonstrated that *Pinus* seeds were negatively affected, but the development of seedlings was not retarded. An explanation could be based on the same essential oils present in *Laserpitium*, *Grafia* and *Pinus*.

5.13.4. Rice straw: allelopathic potential and effect on herbicide efficacy

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Unharvested rice (*Oryza sativa*) is often left in the fields. While preparing their field for next season crop, farmers generally incorporate rice straw (burned and/or unburned) into soil. Incorporation of rice straw into soil may have allelopathic interference to the seedlings of next season crops and adversely influence the performance of soil-applied herbicides. My ongoing research cautioned against certain agricultural practices employed in wheat-rice cropping systems of Northern India. The objectives of the study are to examine: (i) allelopathic potential of rice straw against certain crop species and weed species, and (ii) effect of rice straw incorporation into soil on phytotoxicity of soil-applied herbicides such as isoxaflutole. Results indicate the allelopathic growth suppression of mustard seedlings in soil amended with unburned rice straw; however, effects were eliminated/minimized in soil amended with burned rice straw. Phytotoxic activity of isoxaflutole, a soil-applied herbicide, disappeared when added to soil amended with burned rice straw.

5.13.5. Genetics and biochemistry of the *Sorghum* spp. allelochemical sorgoleone

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Several species of *Sorghum* are known to be allelopathic. The root hairs of *Sorghum* spp. produce and secrete sorgoleone and several structurally related compounds that act as allelochemicals. An EST study was performed for sorghum root hair cells, and putative candidates were found for genes encoding the four types of enzymes expected to be involved in the final steps of sorgoleone biosynthesis: a fatty acid desaturase (FAD), a polyketide synthase (PKS), a P450 monooxygenase (P450), and an O-methyltransferase (OMT). There were three or four candidate genes for each of these enzyme classes, and each was expressed 10- to 1000-fold more in root hairs than in other sorghum tissues. Heterologous expression of candidate FAD, PKS, and OMT open reading frames produced functional enzymes with substrate specificities that suggest a potential role in sorgoleone biosynthesis. The next step will be to determine if sorgoleone biosynthesis can be manipulated by altering the expression of these genes. If so, this will be an important tool in probing the chemical ecology of sorgoleone.

5.13.6. Competition and allelopathy in native and invasive populations of garlic mustard

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To test the hypothesis of higher competitiveness in invasive North American populations of garlic mustard, we set up an experiment containing matrix communities with either European or American species to which garlic mustard of either European or American origin was planted. To distinguish competition from allelopathy, activated carbon (AC) absorbing allelochemicals was added to half of the replicates. Invasive garlic mustard did not grow better than native ones. Instead, plants were adapted to the local community:

American garlic mustard grew better in the American than in the European community, while European garlic mustard grew better in the European than in the American community. But when AC was added, garlic mustard of either origin performed better in their respective foreign community. No direct effect of AC on the growth of any species was found, suggesting that AC suppressed chemical interference among species. These results contradict the hypothesis that invasive populations evolved higher competitiveness. Rather, local adaptation to the respective community highlights the importance of belowground interactions for community structure.

5.13.7. Implications of allelopathy in pest management

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In nature, plants produce allelochemicals which represent a vast diversity of compounds with a variety of biological activity. Such compounds are released directly or indirectly from live or dead plants into the environment through various processes, and may favorably or adversely affect co-occurring plant species. There have been considerable efforts in exploring allelopathic principles in designing alternative strategies in pest management practices. I will illustrate certain examples for the management of weeds, insects, and plant diseases. Use of cover crops and allelopathic crop cultivars in weed management will be highlighted. An attempt will be made to demonstrate if allelopathic crops or cover crops can be used to limit or reduce the use of conventional herbicides. Low activity, high cost, and narrow-spectrum selectivity of allelochemicals may pose limitations in developing successful weed management systems. With current sophisticated molecular and biotechnological tools, the concepts of allelopathy can be better exploited in the future to provide novel pest management programs.

5.13.8. Allelopathy of aquatic photoautotrophs

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Allelopathy, either positive or negative biochemical interactions among plants or between plants and microorganisms (*sensu* Molisch 1937), has frequently been observed in aquatic photoautotrophs, both in marine and freshwater systems. Especially the inhibition of competitors is apparently an adaptive trait for many aquatic cyanobacteria, algae or higher plants. Several allelopathically active compounds have been isolated, yet, unequivocal field evidence for allelopathic interactions is still missing. Frequently the mode of action of active compounds is targeted on photosynthesis or extracellular enzymes, and one compound may affect multiple physiological processes. In the search for allelopathic interactions, proof for the release of active compounds, transfer to and effect on target organisms are required. Environmental conditions (light, nutrient availability) may strongly influence the strength of the interactions. Little is also known about the fate of exuded active compounds in the aquatic environment, microbial degradation, photochemical derivatization or else. Results from our model system *Myriophyllum spicatum* will be presented.

5.13.9. Cyanobacterial defence against grazers: inhibitors of digestive hydrolytic enzymes

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Pelagic and benthic cyanobacteria are heavily grazed by crustaceans, snails, insect larvae, protozoa and other organisms. As in higher plants, physical and chemical defence mechanisms were developed by cyanobacteria to reduce the grazing pressure on them. Screening assays conducted with axenic and monoxenic strains of cyanobacteria showed the wide occurrence of trypsin-, chymotrypsin- and glycosidase-inhibiting compounds. These compounds essentially reduce the digestibility of macromolecules and hence are efficient means to control the development of grazers. The protease inhibitors were cyclic peptides and depsipeptides and the glycosidase inhibitor was an iminosugar. The inhibitors were effective in the nM to μ M range. The efficiency of the inhibitors and the amounts stored in the cyanobacteria are sufficient to essentially reduce the digestion of organisms in the gut of grazer. Even when these cyanobacteria are only minor food constituents, the inhibitory activity is strong enough to suppress digestion.

5.14.1. Tree reaction on stress factors - concept and results of 20 years ICP Forests

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In 1985, the fear of a large scale forest decline in Europe and the uncertain role of air pollution were driving forces to establish the International Co-operative Programme on the Assessment and Monitoring of Air Pollution Effects. Mid of the eighties, mountain forests of the Czech Republic showed a disintegration of forests, decrease in its production functions, failure of its ecological and environmental functions and disappearance of tree species genetic resources. This has had immense ecological and social consequences (PERINA, 1984). An European wide awareness of forest condition issues was generated. Tree reaction on stress factors is a core concept of ICP Forest, the largest biomonitoring programme worldwide. The Ministerial Conference on the Protection of Forests in Europe (MCPFE) has chosen defoliation as an indicator for forest health and sustainable forest management. Today's ICP forest Level 1-concept on the assessment of visible tree condition fulfills the ecological requirements to evaluate tree vitality. Quality assurance has been a matter of particular concern in the biological part of the ICP Forest programme. In order to detect, discuss and manage differences in methods applied by the countries inter-calibration courses are being offered annually. As an additional measure standardized photos of tree crowns were circulated by postal mail. The digital image analysis system CROCO gives reference values for each photo. The health of forests may be heavily influenced by biotic agents or pathogens, such as insects or fungi. Therefore, in 2004 the submanual of crown condition assessment has been revised substantially.

5.14.2. Nutritional state of trees monitored in ICP Forests programme

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The nutritional status of trees often indicates specific processes in the ecosystem. Inadequate nutrient supply may be a direct cause of low tree vitality or a factor that increases adverse effects of air pollution. High concentrations of certain elements in foliar tissues may be the effect of intoxication or of high air-pollution levels. Foliar sulphur concentrations followed annually since 1987 on 36 Finnish and 71 Austrian ICP Forests Level I plots have been low throughout the monitoring period. Even at this low level, the proportion of trees in the medium and high sulphur concentration classes have decreased reflecting the drastic reductions in sulphur emissions from 1980's to the present. Generally foliar N concentrations in these Finnish and Austrian plots have remained low especially in Austrian forest located in Alpine regions. Nutrient ratios detected in the monitored trees have indicated generally well-balanced nutritional status. Considering the whole Europe in half of the 109 Level II plots, which provided necessary information for calculations, unbalanced nutrition caused by high nitrogen deposition have been observed.

5.14.3. Biodiversity state and monitoring of forest ecosystems in Europe: the ICP Forests initiatives

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In the frame of the ICP Forests biodiversity test-phase and anticipating the new tasks required by Regulation (EC) n. 2152/2003 *Forest Focus*, a first biodiversity survey has been carried out in some selected Level II EU/ICP Forests permanent plots in 2004 and 2005, in the framework of the Pan-European project *ForestBIOTA*, joint project by 14 European Countries, based on 123 EU/ICP Forests permanent plots. The key parameters, assessed at stand level, include (1) vegetation, (2) epiphytic lichens, (3) stand structure, (4) deadwood, (5) insects communities, (6) naturalness and (7) landscape biodiversity. Methods for field surveys follow that adopted by the ICP Forests Working Group on Biodiversity in 2003. First results show that: (1) in very short time and relatively not expensive costs is possible obtaining valuable indications on biodiversity status of forest communities; (2) the harmonized methods are reliable and effective; (4) the surveyed forest communities have shown very high values for nature conservation; (5) qualitative results of

surveys are very important to increase the basic scientific knowledge.

5.14.4. Plant diversity as an aspect of forest condition in the UNECE ICPForests programme.

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Intensive monitoring plots of the ICPForests gathered an amount of data about the ground vegetation in forest ecosystems throughout Europe. Each Country, applying different field techniques, conform to common rules of procedure, under the suggestions of a dedicated Expert Panel which implemented a Unified Coded Flora and comparability targets. Data series are foreseen to contribute to: definition of the forest ecosystem state and changes evaluation; assessment of the specific plant diversity at the ecosystems level. The contribution to scientific knowledge and to Global and Pan-European biodiversity initiatives and networks (ICP-IM, MCPFE, CBD, Forest BIOTA, ALTER-net, etc.) are also underlined. In spite of site-related data, first results (more than 670 plots, with large differences in plant diversity) depict the linkages with temperature, precipitation, dominant tree species and actual soil acidity. Nitrogen deposition seems to have some significant influence, which claims to further studies. Plant data series from ICPForest's plot, can be used for on-site confirmation of models including biodiversity k-factors and environment relations.

5.14.5. Effect of environmental pollution on Scots pine stands in Poland: an eleven year survey of mycorrhizal coenosis

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Ectomycorrhizal fungi play an essential role in the forest community and anthropogenic emissions that modify fungal diversity may have ecologically and economically far-reaching effects on forest productivity. The ectomycorrhizal coenosis of Scots pine (*P. sylvestris* L.) were studied in order to determine how long-lasting but declining environmental pollution (acidification, increased aluminum availability, HF), influence mycorrhizal community structure and growth of *P. sylvestris* stands. Three different approaches were performed: sporocarp inventories, morphological analysis of ectomycorrhizal roots and molecular techniques based on PCR-RFLP method. Over eleven growing seasons (1992-2002) significant changes was observed in above- and below ground communities of ectomycorrhizal fungi between both tested sites. We will use this data to address the question "does fluctuation in mycorrhizal coenosis affect tree growth and biomass of Scots pine?".

5.14.6. A transect of vegetation and soil along a moist temperate coniferous forest

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The floristic variation in Asian temperate coniferous forests is poorly understood. Species composition may be either unpredictable as abundance fluctuate randomly, more or less uniform, or it may correspond to environmental heterogeneity. We tested the three hypothesis by studying the floristic variation along a transect that crossed a mixed coniferous forest in Pakistan. Vegetation Data were simplified by Cluster Analysis, Detrended Correspondence Analysis (DCA) and Spearman's Rank Correlation Coefficient was used to detect relationship between environmental factor and species distribution. Soils were physically and chemically analyzed. Plant diversities and assemblage with respect to environmental features in these forests were discussed. Results suggested that substrate and tree density were significantly associated with the vegetation variation along the altitudinal gradient.

5.15.1. Implementing the GSPC at national level - progress, problems and opportunities.

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At the sixth Conference of Parties to the Convention on Biological Diversity, the delegates adopted the Global Strategy for Plant

Conservation including sixteen outcome-oriented targets. The Parties emphasized that the targets should be viewed as a flexible framework within which the national and/or regional targets may be developed, according to national priorities and capacities, taking into account differences in plant diversity between countries. Further, Parties were invited to develop national and/or regional targets, and as appropriate, to incorporate them into relevant plans, programmes and initiatives, including biodiversity strategies and action plans. However, whilst major efforts have been made at international level to implement the strategy, progress at national level has been slow. The GSPC present a strategic opportunity for the botanical community to contribute to national level processes through innovative linkages and partnerships. The paper discusses the progress in implementing the GSPC at national level, challenges and opportunities and presents potential options.

5.15.2. What does the GSPC require from the world's botanists?

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The GSPC presents plant diversity specialists with a unique opportunity to connect their work with an internationally agreed priority under the Convention on Biological Diversity, and thus to assert their relevance. However, this privilege comes with a responsibility to make real progress against the 16 outcome oriented targets before 2010. Failure to rise to the challenge will be negative for the future of plant diversity, and also for the future development of our field. Progress will require urgent and concerted action, as well as broad participation among the international botanical community. All of the targets are important, but target 1 - the completion of a widely accessible working list of known plant species - is fundamental. Success in this area will be crucial for progress with many of the other targets. It will also be of great value scientifically, allowing more effective integration of the mass of data already accumulated by centuries of scientific work.

5.15.3. The GSPC and its relationship to the activities of the IUCN Species Survival Commission

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 Tropical Botanic Garden, Miami, FL, United States, ³Christchurch
 Botanic Garden, Christchurch, New Zealand.

The Species Survival Commission (SSC) of IUCN--the World Conservation Union regards the Global Strategy for Plant Conservation (GSPC) as essential for plant conservation. SSC, the world's largest established network of conservation professionals, includes scientists and managers effectively spanning *in situ* and *ex situ* communities. A key product is the IUCN Red List of Threatened Species, and SSC is focussing on improving knowledge of plant conservation status in the Red List (GSPC target 2). With Plantlife International, IUCN is working on identification and conservation of Important Plant Areas (GSPC target 5). The strength of SSC's global conservation network comprising over 1000 botanists in 35 Specialist Groups, linking with IUCN's 1000+ membership of Governments and NGOs, is to compile experience and produce professional manuals and protocols to help plant conservation practitioners and policy-makers throughout the world. Botanists are the fundamental link if the ambitious targets in the GSPC are to be achieved by 2010, and SSC is a mechanism in which IBC participants may contribute to ensure that the plants they study remain for future generations.

5.15.4. Building capacity to implement the GSPC - the Southern African experience

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Southern Africa possesses an extremely rich flora (ca. 30 000 taxa) in ecosystems ranging from desert to rainforest. In 1994, the ten countries of the region established a capacity building network (SABONET) with strong support from national governments and donor agencies. SABONET has contributed significantly to reaching GSPC targets 1, 2, 3, 5, 8, 14, 15 and 16. Training has included 134 in-service trainees in 12 countries, 26 post-graduate fellowships receiving 36 higher degrees, 63 travelling internships between institutions in the region, 3 regional collecting expeditions, electronic databasing of 450 000 herbarium specimens in 17 herbaria, national checklists published for Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, Swaziland, Zambia and

Zimbabwe; Red Data Lists for all ten countries, publication of a series of comprehensive needs and resource analyses, and regular publication of *SABONET News*. The SABONET project has been underpinned by extensive training and development programmes of the South African National Biodiversity Institute that has focussed on user needs based human resource development over the last decade.

5.15.5. New initiatives from botanic gardens to implement the Global Strategy for Plant Conservation

S. L. Sharrock;

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The global botanic garden community, working together with Botanic Gardens Conservation International (BGCI), has been closely involved in the development of the Global Strategy for Plant Conservation, providing key support for its development following the XVI International Botanical Congress in 1999 and campaigning for its ultimate adoption by the CBD in 2002. This community is now fully committed to the implementation of the Strategy, as demonstrated by the recent adoption of global botanic garden 2010 targets directly related to the GSPC targets. Botanic garden networks around the world are also developing 2010 targets at national and regional levels. Examples of these targets are provided. BGCI, through the HSBC-funded Investing in Nature programme, is supporting implementation of the GSPC, both through in-country programmes with botanic gardens and through support to the CBD Secretariat. New BGCI in-country programmes focus on both conservation and education activities and are being implemented in countries in Asia, North, South and Central America, the Middle East, Africa and Russia. Examples of these programmes are provided.

5.15.6. Developing and implementing a national plant conservation strategy in Colombia

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The Colombian Strategy for Plant Conservation was created five years ago by 18 private and public organizations. The result of its work was an ambitious but realistic document outlining a plan to conserve Colombia's hugely diverse flora. The five thematic axes included in the strategy are investigation, monitoring and handling of the information, conservation in situ and ex situ, use and handling of the plants, education and public understanding and invigoration and inter-institutional cooperation. However the results have been not yet been as positive as we hoped they would be. Many native plants are disappearing and so now Colombian institutions are starting a large multi-stakeholder project, to save 2,500 threatened plants of Colombia. We need international cooperation to achieve the project aims.

6.1.1. How do plants cope with abiotic stress?

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Abiotic stresses especially drought and salinity are the primary causes of crop loss worldwide. To maintain growth and productivity plants have developed adaptive mechanisms to tolerate stress. Drought, salinity and extreme temperature are often interconnected and can lead to similar cellular damage. As a consequence, similar signalling pathways are activated.

This presentation will focus on dehydration stress. Desiccation-tolerance, the ability to recover from almost the complete loss of cellular water, is part of the normal developmental programme of higher plants and is acquired during seed maturation, but lost during germination. Resurrection plants are a small group of plants which tolerate desiccation in vegetative tissues. Resurrection plants are an excellent experimental system to investigate the molecular basis of desiccation tolerance in vegetative parts of plants. The molecular basis of desiccation tolerance will be discussed in comparison to dehydration responses in non-tolerant plants and under evolutionary aspects.

6.1.2. Genetic and molecular dissection of adaptive responses to progressive drought

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Leung;

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Drought adaptation involves dynamic adjustments of many cellular processes including gene expression profiles to modifications of growth patterns to optimize water usage. These processes are known to be regulated by ABA-dependent and ABA-independent signalling pathways. The most robust and immediate response is stomatal closure to limit water loss through transpiration (>95% of the water loss). Transpiration cools the leaf surface and the ensuing changes in temperature can be monitored by remote infrared thermography. Our laboratory has exploited this non-invasive technology to systematically isolate mutants that fail to close the stomatal pore as a means to identify genes specifically mediating adaptive responses to progressive drought. Among mutations belonging to six complementation groups, we have cloned the corresponding genes for two of them. One is the OPEN STOMATA 1 (OST1) which encodes an homologue of the AMP-activated kinase family. The OST1 activity is stimulated by low humidity and exogenous application of ABA. To understand its role in a biological context, our recent effort has been directed toward identifying downstream targets.

6.1.3. Functional Genomics of the heat stress transcription factor (Hsfs) family of *Arabidopsis*

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Heat stress transcription factors (Hsfs) are the major regulators of the plant heat stress response. Sequencing of the *Arabidopsis* genome revealed the existence of 21 ORFs encoding putative Hsfs assigned to classes A, B and C. Here we present results of a functional genomics approach focused on the analysis of their C-terminal domains harboring conserved modules for their function as transcription factors and their intracellular localization. Short peptide motifs enriched in aromatic and large hydrophobic amino acid residues embedded in an acidic surrounding (AHA motifs) are essential for transcriptional activity of class A Hsfs. We provide also evidence for the function of a leucine-rich region at the very C-terminus as a nuclear export signal (NES) of class A Hsfs. Microarray analysis demonstrates that all 21 *Arabidopsis* Hsfs are expressed on the transcript level which is changed specifically in response to a variety of environmental stresses and/or developmental signals. This indicates rather specific than redundant functions for each Hsfs being integrated in diverse signalling pathways.

6.1.4. A Role for glycogen synthase kinases in plant stress signaling

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Plants encounter various environmental stresses. Plants respond to these threats with an array of events starting with signal perception, followed by subsequent intracellular signal transduction to finally induce a proper physiological response. Protein phosphorylation is pivotal for intracellular stress signaling. Members of the family of glycogen synthase kinase 3 (GSK-3) regulate numerous signaling pathways and homologues can be found in all eukaryotes. We are focusing on the role of GSK-3/Shaggy-like kinases (GSKs) during plant stress signaling. The activity of distinct alfalfa GSKs is modulated during the immediate response to different stresses. Wounding activates WIG (wound-induced GSK). Treatment of plant organs with cellulase or the herbicide paraquat regulates Msk1 activity levels and overexpression of Msk1 affects plant stress tolerance. Genetic and biochemical data indicate that Msk4 plays a role in salt stress adaptation. Msk4 activity is induced by high salinity. Overexpression of Msk4 enhances plant resistance to high salt stress and modulates the levels of specific metabolites.

6.1.5. Characterization of the calcium dependent protein kinase CPK3 from *Arabidopsis*

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Calcium signaling is a central mechanism in plants to regulate responses to diverse environmental stimuli. Plants have evolved a special group of Calcium dependent protein kinases, the CPKs. 34 CPK genes were predicted from the genomic sequence of *Arabidopsis thaliana*. We have started to characterize the Calcium

dependent protein kinase CPK3 (At4g23650) from *Arabidopsis*. We used expression of YFP fusion proteins in *Arabidopsis* leaf protoplast to analyze its subcellular localization. In contrast to the results of Damann et al. (2003), who studied the localization in roots, we observed a localization to membranes and the chloroplast. Furthermore, we analyzed *cpk3* knockout mutants and 35S::CPK3 overexpressor plants, expressing either the wild type or a loss-of-function allele of CPK3, for phenotypes under different growth conditions. In order to isolate potential CPK3 targets yeast two-hybrid screens were performed. In addition we used transient transformation of *Arabidopsis* protoplasts to study activation of CPK3 kinase activity in response to various external stimuli.

6.1.6. The two strategies of plants to cope with chronic low dose irradiation

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The adaptive responses of plants to an increase in radioactivity of environment were established in radiobiological researches carried out in the Zone of the Chernobyl Nuclear Power Station. It has been shown that there are two types of adaptive strategies to the environment contaminated with radionuclides, namely ontogenetic and phylogenetic strategies. The ontogenetic adaptive strategy is attained by induction of DNA repair. Increasing of the genetic variability in irradiated population performs the phylogenetic adaptive strategy. The mechanism of this strategy is associated with induction of genetic instability. Rise in the frequency of genetic recombination as well as heterozygosity is at the time essential components of phylogenetic adaptation. Ontogenetic and phylogenetic adaptation strategies are an important feature in coping of plants to the elevated chronic irradiation. Most likely plant cells as a stress signal into adaptive responses perceive the low doses of irradiation.

6.2.1. The use of genomic tools to examine the responses of *Chlamydomonas reinhardtii* to its environment.

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We have used cDNA- and oligonucleotide-based microarrays to explore the acclimation of the unicellular, green alga *Chlamydomonas reinhardtii* to changes in both light intensity and quality, and to sulfur and phosphorus starvation. In one study, we have investigated the role of the PHOT1 photoreceptor in blue light regulation of gene expression and in the coordination of genes encoding LHC polypeptides and enzymes needed for chlorophyll and carotenoid biosynthesis. Extensive work has also been performed on sulfur and phosphorus limitation. While nutrient deprivation strongly influences functions involved in scavenging the limiting nutrient (e.g. expression of specific transporter genes), starved cells must also stop division, arrest growth and control metabolic processes. Sulfur and phosphorus deprivation caused dramatic changes in the activities of genes encoding proteins involved in acquiring and utilizing sulfate and phosphate, respectively, as well as in the activities of genes involved in key metabolic processes such as photosynthesis. Several mutants have been isolated that are not able to acclimate to nutrient deprivation; many of these strains die in the light as nutrient levels decline. This 'death' phenotype may reflect the inability of these cells to properly coordinate aspects of the photosynthetic electron flow (and the elimination of excitation pressure) with the potential for cell growth. My talk will focus on the use of genomic technologies to dissect acclimation responses of *C. reinhardtii*, and the results acquired with these technologies will be discussed with respect to the physiology of photosynthetic organisms under changing environmental conditions.

6.2.2. Extracellular thiol protease sensitizes the dinoflagellate *Peridinium gatunense* to oxidative stress thus synchronizing their cell death

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The phytoplankton population of Lake Kinneret is characterized by an annual winter-spring bloom of a dinoflagellate, *Peridinium*

gatunense. We showed that CO₂ limitation initiate its collapse within a couple of weeks in early summer. This is mediated by oxidative stress and activation of cysteine proteases. Aging of the *Peridinium* cells resulted in DNA fragmentation and enhanced sensitivity to oxidative stress. This was accompanied by a rise in the intracellular and extracellular specific protease activities. Exposure of young *Peridinium* cells to media from older cultures enhanced their sensitivity to oxidative stress, and led to massive cell death, suggesting presence of a conditioning factor in the spent media from old cultures. Inhibition of protease activity in this media suppressed the sensitivity to oxidative stress and the resulting H₂O₂-induced cell death. In contrast, addition of papain, to the young cultures increased cell death. We show that a protease excreted by the older cells sensitizes the younger cells to oxidative stress, thereby coordinating the crash of the entire *Peridinium* population, young and old cells alike, at the end of the bloom.

6.2.3. In-situ physiology of algae by means of single cell techniques

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The primary production in the aquatic environment is basically influenced by the availability of light and nutrients and the physiological activity of the phytoplankton community. The physiological activity of the phytoplankton cells depends on the genetic diversity of the cell assemblage and on the acclimation state of the species. Biodiversity can be measured by means of microscopic observation or via molecular tools, whereas physiological parameters can be measured only on the community level. Recent progress in single cell techniques allows to collect detailed physiological information with a rather high taxonomic resolution. It is shown how the combination of in-situ hybridization with flow cytometry, absorption, fluorescence and FT-IR spectroscopy can be used to quantify the contribution of different taxa to carbon acquisition and nutrient usage.

6.2.4. Iron uptake and homeostasis in marine diatoms

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Iron (Fe) is an essential element for all organisms, including oceanic phytoplankton. However in the open ocean, the concentration of dissolved Fe is low (10-400 pM) since the main redox species, Fe(III) is poorly soluble at seawater pH. Mesoscale *in situ* Fe addition experiments in high nutrient, low chlorophyll regions have demonstrated that diatom production is limited by Fe availability. Diatoms are particularly important because of their ability to promote the oceanic sequestration of CO₂ through enhanced export of diatom biomass below the euphotic zone. Despite the importance of Fe for diatom growth, we know little about Fe uptake systems or Fe-regulated genes in diatoms. The sequenced genome of *Thalassiosira pseudonana* identified Fe uptake systems similar to those found in yeast. Data on the expression of Fe-regulated genes as a function of dissolved Fe concentration combined with physiological measurements are used to characterize the survival response to Fe limitation.

6.2.5. High affinity iron transport in *Chlamydomonas reinhardtii*

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Using electrophoretic separation and mass spectrometric analyses, we have identified plasma membrane polypeptides, frequently of unknown function, whose synthesis and/or degradation were altered under Fe deficiency. In some cases however, the expression of the corresponding genes to these unknown proteins was regulated by Fe in a similar manner to FLP (Fox1). Fox1 encodes a ferroxidase and is known to participate in Fe uptake. RNAi analysis using a portion of Fox1 resulted in greatly reduced transcript for Fox1 and synthesis of Fox1p. However, high affinity Fe transport was reduced by only ca. 50%. In addition, a membrane protein was identified that was synthesized de novo under Fe deficiency and showed distinct sequence homology to Fe(III)-chelate reductases (e.g. Fro2 and FRE1). Although the mechanism of Fe uptake at the plasma

membrane of *Chlamydomonas* resembles *Saccharomyces*, there are also likely other high affinity transport systems present.

6.2.6. Channelrhodopsin, a hybrid light sensor in green algae

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The green alga *Chlamydomonas reinhardtii* contains two mammalian type rhodopsins with so far unknown function and 5 microbial type rhodopsins. Two of the latter, Channelrhodopsin-1 (ChR1) and Channelrhodopsin-2 (ChR2), exhibit light-gated ion channel activity after expression in *Xenopus oocytes* (1,2). ChR1 is highly selective for H⁺ whereas ChR2 conducts H⁺, Na⁺, K⁺ and Ca²⁺. Upon stimulation with light pulses, the photocurrents of both channelrhodopsins partially inactivate before a stationary current is observed, very similar as observed for photocurrents recorded from *Chlamydomonas* eyes. Recovery in the dark is slow but accelerates at negative membrane voltage and low extracellular pH. We have developed a complete model that quantitatively explains light dependence and kinetics of both rhodopsins. Due to the extremely fast activation of the conductance we have proposed that both channelrhodopsins are mediating phobic responses. This conclusion was supported by action spectroscopy for fast photoreceptor currents in *C.reinhardtii* at neutral and alkaline pH and is compatible with electrical measurements on antisense transformants with reduced content of ChR1 (3). The contribution of both ChR1 and ChR2 to phototaxis, especially at low light is still unknown. The expression of the ChR1 and ChR2 is light dependent and possibly under circadian control. Recently, we have found three more microbial rhodopsin sequences in the Genbank of *C.reinhardtii*, which we named Cop5, Cop6, Cop7. All three are coupled to transducer sequences and might be part of a microbial type "two component signaling system". These new rhodopsins are photoreceptor candidates for phototaxis at low light. 1. Nagel, et al. (2002) *Science* 296, 2395-2398, 2. Nagel, et al. (2003) *PNAS* 100, 13940-13945, 3. Sineshchekov et al. (2002) *PNAS* 99, 8689-8694.

6.2.7. Organelle interactions and possible degradation pathways in the green alga *Micrasterias* visualized by energy filtering transmission electron microscopy (EFTEM)

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Organelle and vesicle interactions are essential for both anabolic and catabolic pathways in plant cells. In this study we investigate structural details of different organelle-organelle, organelle-vesicle and organelle-membrane interactions in *Micrasterias denticulata* cells of different age, by use of high pressure freeze fixation and energy filtering transmission electron microscopy (EFTEM). Contacts between organelles are achieved by formation of a cone-shaped protuberance by one of the compartments which penetrates into its fusion partner. In the same way lytic compartments contact organelles and vesicles by extending their contents into the respective compartment. Detached portions of organelles are found inside lytic compartments as a consequence of such interactions. Fusions between different populations of mucilage vesicles may represent a fast excretion machinery. Partial disintegration of mitochondria upon contact with the plasma membrane point towards a release of these organelles. All organelle, vesicle and membrane contacts observed, occur independently from cytoskeleton function as demonstrated by use of inhibitors.

6.3.1. Root growth, nutrients, soil organisms and soil structure

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Roots acquire nutrients from soil and return nutrients to soil as exudates, sloughed cells and decomposing tissue. These processes involve close interaction between roots and rhizospheres, from arrival of a root in a soil volume to its death and decay. Interactions rely on kinematics, soil structure, including the propensity of roots to grow where others have grown before, and dynamics of soil organisms. They have substantial agronomic implications. Here we use wheat to illustrate the principles. Root

apices extend rapidly for nutrients or water unless hindered by soil structure or temperature. Extension rates affect root hairs and branches, and rhizosphere microbes via exudates. Remnant roots and rhizospheres provide pathways and inocula that help or hinder new roots. Wheat genotypes vary in growth rates, exudates, and responses to the structure and microflora of unploughed soils. Those adapted grow roots quickly and host less deleterious bacteria and fungi, due in part to residence time on root caps. We use fluorescence *in situ* hybridization of microorganisms on field roots, *in situ* elemental analyses, and time-lapse imaging to quantify these interactions.

6.3.2. Variations in developmental pattern of lateral roots induced by environmental factors: the response to mechanical stress induced by slope.

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We show that modifications of root architecture induced by the mechanical stress acting on slope cannot be explained only with the simple reiteration of an intrinsic pattern of lateral root formation which is independent from root-category and -function. Therefore we suggest that in an adult root system, the development of new lateral roots might follow also a different pattern in response to specific environmental signals perceived by the plant. Our findings show that pericycle cells cannot be the only source of initials for lateral root primordial morphogenesis and propose that new ray initials from the cambium might be engaged to provide the source for new root meristem initials. The contemporaneous activity of these two developmental patterns explains why it is impossible to determine in the root system of adult plants the existence of growth units analogous to the phytomers responsible for stem development.

6.3.3. Role of ABA in root growth maintenance under water deficits: prevention of high levels of reactive oxygen species

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Accumulation of abscisic acid (ABA) is required for root growth maintenance under water deficits in maize seedlings (Sharp 2002, *Plant Cell Environ* 25: 211-22). To investigate the hypothesis that ABA functions in regulating the antioxidant system to maintain reactive oxygen species (ROS) at non-damaging levels, effects of ABA deficiency in the root growth zone are being studied using the vp14 mutant, fluorescent imaging of ROS levels, high resolution kinematic analysis of elongation rate profiles (van der Weele et al., 2003, *Plant Physiol* 132: 1138-48), and microarray analysis of gene expression. Under water deficits, ROS levels increase slightly in wild-type roots and dramatically in vp14. The effect of ABA-deficiency is specific to the apical 1-3 mm where cell elongation is normally maintained under water deficits but is inhibited by ABA deficiency. Loss of membrane integrity occurs in the same region of the ABA-deficient roots. Time course experiments indicate that increase in ROS precedes and causes the loss of membrane integrity. Broader implications for the role of ABA in stressed plants will also be discussed. NSF Plant Genome Program, DBI-0211842.

6.3.4. Longitudinal pattern of lateral root initiation: predictable or not predictable? A case study of *Arabidopsis*

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Lateral root (LR) initiation is considered to occur in an acropetal pattern. We analyzed the longitudinal pattern of LR primordium (LRP) initiation along the primary root in *Arabidopsis* using two approaches. First, we estimated the density of LRs as a ratio between the number of LRs and the length of the LR-bearing root portion. This approach permitted the evaluation of how LR density is related to the average number of cells between LRPs. We found that in younger roots, the LRP density was similar to the LR density in the region of the root bearing LRs and that in older roots, the LRP density in this zone decreased. The dilemma of delayed or de novo LRP development will be discussed. Second, we estimated the inter-primordial distance expressed in units of length and in the number of pericycle cells. Our data apparently indicate that LRP initiation obeys a pattern of regular spatial distribution, possibly established by inhibition via signals produced by already

initiated primordium. This demonstrates that the developmental principle of lateral inhibition may operate during LRP initiation. Acknowledgements: DGAPA, IN 210202 (UNAM), FOSEMARNAT-2004-01-80.

6.3.5. Rhizosphere nitrogen and root development in *Arabidopsis*

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Like the rest of the plant, the root system has no pre-defined body plan, rather its development is continuously modified by interactions with environmental factors, including nutrients. We have been studying the role of nitrate in regulating root branching. A MADS box transcription factor (ANR1) is known to be required for the stimulation by nitrate of meristematic activity in the lateral root tip. Current studies are focussed on understanding how ANR1 acts and identifying its downstream targets. We have recently obtained evidence that roots are also capable of sensing and responding to the presence of organic nitrogen in the soil. *Arabidopsis* root growth was found to be highly sensitive to the presence of external glutamate, but not to the majority of other amino acids. The ability to sense glutamate appears to reside in the root tip itself and is common to both primary and lateral roots. We will discuss our current understanding of the genetic and physiological basis of this phenomenon and its possible relationship to the existence of a family of glutamate receptor genes in plants.

6.3.6. Cytokinin regulates root development and gravitropism

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In roots, cytokinin is mainly produced in the cap. From there, cytokinin is exported upward through the xylem and accumulates in young shoot organs with highest transpiration. Cytokinin has a negative regulatory role in root growth and might function as an inhibitor of tropistic root elongation during early phase of gravity response. In vertically growing *Arabidopsis* roots the cytokinin in the cap is distributed symmetrically. When roots are turned to a horizontal position, free cytokinin is transported laterally within less than 30 min and becomes concentrated at the new lower side of the cap, where it locally retards elongation of the lower side and promotes elongation of the upper root side. This asymmetrical activation pattern causes the initiation of a downward gravitropic root bending near the root apex. Exogenous application of cytokinin to vertical roots induced bending toward the site of application, indicating that cytokinin has an inhibitory effect in root gravitropism. Our results suggest that early root gravireponse is controlled by cytokinin. We conclude that both, cytokinin and auxin are key hormonal signals which regulate root gravitropism.

6.4.1. Can the geographical distribution help to understand the function of growth forms?

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There are different functional approaches of growth forms: First, the study of the plants in the plant community and succession, e. g. the space conquering (phalanx and guerilla), the competitive ability (c, r, s strategy), the relation to herbivory, and resource partitioning. Second, to investigate the relation to local site conditions. Here, statistics show the adaption e. g. of runners to sandy or wet soil, of taproots to deep, dry soil. Finally, it is also possible to study the worldwide distribution of growth form characters. The distribution patterns are either connected with the geographical distribution of site conditions (e. g.: root sprouting on bare, eroded, often dry soil; pseudo-annuals in the dark taiga with rare light spots) or with the regional climate, especially with the length and the conditions of the growth period. There are some published examples (stem succulents, thorny cushions, lianas). Some others will be discussed in detail: bulbs and tubers, rosulate/erosulate and monopodial/sympodial growth. This "Raunkiaerian approach" reveals evolutionary advantages of the special character beyond mere speculations on the function.

6.4.2. Bud morphology and shoot growth dynamics in two species of Mediterranean sub-shrubs co-existing in gypsum outcrops.

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Understanding the effects of the environment on the morphology and shoot growth activities of plants is crucial to identify plant ecological strategy. This study analysed the bud morphology, bud activity, shoot growth dynamics and shoot water content at full hydration (WC_h) of two species of Mediterranean sub-shrubs, *Lepidium subulatum* and *Linum suffruticosum*, co-existing in gypsum outcrops in NE Spain. Both species bore naked buds and displayed discontinuous seasonal patterns of shoot growth, leaf primordia formation and WC_h as a result of the strong seasonality of Mediterranean climate in gypsum outcrops. The number of leaf primordia in the bud peaked before the beginning of shoot expansion. In both species, organogenesis and expansion were uncoupled throughout the year. The time lapse between these two processes varied throughout the year, and was greatest for those elements differentiated in autumn. The beginning of the spring period of expansion of long branches coincided with maximum values of WC_h , while the rest period of summer matched minimum values. These results support the hypothesis that the growth of long branches and WC_h are strongly related.

6.4.3. Finding the Center of a Phyllotactic Pattern

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An important quantity characterizing a phyllotactic pattern is the divergence angle, i.e. the angle between primordial that form consecutively. This angle often varies only slightly about some particular value as the plant develops. The calculation of divergence angles between primordia in shoot apical meristems (SAMs) depends on the point used as the center of the SAM. The field of phyllotaxis has been challenged by the diversity of shapes that SAMs can have. In mathematically ideal phyllotactic patterns, the center is well defined but there has not been a precise definition for the center of naturally occurring phyllotactic patterns. A few techniques have been proposed for estimating the location of the center but without a precise definition for the center the accuracy of these methods cannot be analysed. In this talk I will present a precise definition that can be used as the center of a phyllotactic pattern and a numerical method which can accurately find this point. This helps lay the groundwork to objectively compare theory against experiment for a more general class of phyllotactic patterns.

6.4.4. Plant architecture of Gentians - a source for taxonomy and evolutionary biology

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The genus *Gentiana* L. is rich in species and in different growth forms as well. Although some authors already stressed the relevance of detailed growth form characters for the taxonomy of Gentians, such traits are not yet fully implemented in the common taxonomical concepts. Even in the newly published worldwide revision of the genus *Gentiana* the authors did not utilise this character set with its full accuracy of discrimination. Despite of the low number of well dissected specimens in herbariums and the low availability of fresh material in many taxa, we provide an overview on crucial growth form characters that should be used to achieve a better resolution of the subgeneric classification of Gentians. Besides the already accepted value of basic architectural traits like monopodial or sympodial branching we emphasise the taxonomical power of further morphological characters (i.e. leaf dimensions, longevity and longitudinal dispersion; root system) for inter and intraspecific classification and evolution processes in *Gentiana*.

6.4.5. The rare and valuable species of the sort of *Gentiana* L. and the methods of their preservation

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As a result of worsening ecological situation, increasing of anthropogenic influence the area of the rare, valuable species of plants has been much narrowed that caused to the threat of their disappearing. The reintroduction is one of the methods of preservation of these species in nature. During 1998-2000 in the Botanical Gardens of the Ivan Franko Lviv National University carried out investigations of the rhythm of growth and development of *Gentiana lutea*, *Gentiana cruciata*, *Gentiana punctata*. As a result of investigations was estimated that these species have the stable rhythm of development in the conditions of introduction. The last is evidence of the good adaptation of these species. Reproduction is going on the generative or vegetative ways. The optimal date of the seminal sowing are October, planting - August-September. Sowing seeds in spring it is necessary to make stratification during 60-90 days. There is perspective the reproduction of these species of the sort of *Gentiana* under the conditions in vitro because they are micorism and characterized by longtime generative cycle.

6.5.1. Shoots and leaves, an evo-devo perspective

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The Class I Knotted-like homeobox (KNOX 1) genes are highly expressed in the shoot apical meristem but not expressed in the emerging leaf primordium in tobacco, maize, or *Arabidopsis*. We have analyzed compound leaf producing shoot apices in clades with independently derived compound leaves and shown that with one exception (a derived clade in the Fabaceae) compound leaves always show expression of KNOX genes (Bharathan et al., 2002). In the derived pea clade the LFY/FLO gene regulates this function of generating leaf complexity. We have explored the function of LFY in the basal and derived clades in the Fabaceae. In addition, we find that other genes like PHANTASTICA might play a role in determining the form of the compound leaf generated. Global gene expression differences between simple and compound leaves are also being analyzed using *Neobeckia aquatica* RNA on the *Arabidopsis* gene chip. In addition, we are exploring the evolution of KNOX genes and their role in shoot morphology across Angiosperms. This research is funded by the National Science Foundation.

6.5.2. Evolution of morphological flexibility in Lentibulariaceae

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The Lentibulariaceae (Lamiales) is the largest carnivorous plant family and has undergone an extraordinary degree of adaptive radiation, with unprecedented morphological and ecological diversity. The bladderwort lineage (*Utricularia* and *Genlisea*) is substantially more species-rich, phenotypically divergent, and variable in molecular evolutionary rates than its sister lineage, the butterworts (*Pinguicula*). Bladderworts have a relaxed body plan, characterized by apparent rootlessness and indistinct leaf/shoot organogenesis, that has permitted the evolution of terrestrial, epiphytic, lithophytic, and highly varied aquatic forms that capture prey in intricately-designed suction bladders or corkscrew-shaped lobster-pot traps. In contrast, the flypaper-trapping butterworts maintain vegetative structures more typical of angiosperms. The underlying evolutionary mechanisms responsible for the extreme developmental flexibility found in the bladderwort lineage remains elusive. However, future developmental-genetic approaches that target hormonal regulatory pathways, may provide some answers.

6.5.3. Investigations into the genetics and evolution of unusual growth forms in phyllomorphic *Streptocarpus*

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The genus *Streptocarpus* is morphologically highly labile and some species exhibit a drastically reduced plant body. While species belonging to subgenus *Streptocarpella* possess a 'traditional' shoot apical meristem (SAM) and ordinary serial phytomer development,

species of subgenus *Streptocarpus* include predominantly unifoliate and rosulate taxa and lack a conventional SAM. Unifoliate taxa possess a single 'phyllomorph' made up of a petiolode, lamina and basal serial inflorescences. Rosulate taxa possess several phyllomorphs. The switch between unifoliate and rosulate morphologies occurred several times in the evolution of the genus. We demonstrate the involvement of developmental genes such as SHOOTMERISTEMLESS (STM) in generating the phenotype of these different growth forms. Moreover, we use gene expression patterns to understand the origin and development of meristems in this complex phyllomorph system.

6.5.4. Unusual organogenesis of *Monophyllaea* and *Guarea* from view point of developmental genetics on leaf morphogenesis of *Arabidopsis thaliana*

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Guarea spp. and *Monophyllaea* spp. are unusual in having indeterminate leaves. *Guarea* species of Meliaceae have a shoot-apical-meristem (SAM)-like apical meristem (LAM) on the tip of their compound leaves. We found that *Guarea* LAM expresses high level of mRNA of *LEAFY* homolog as much as in the SAM, while primordia of leaflets do not, suggesting that LAM has a part of natures of SAM. On the other hand, the indeterminate leaf of *Monophyllaea* species of Gesneriaceae, called "phyllomorph", is composed of a single leaf lamina and a petiole. A phyllomorph continues to expand the existing leaf lamina by a special meristem in the basal part of the leaf lamina. In dicots, cell proliferating activity is gradually diminished from the apical part of leaf primordia and later stages of leaf expansion are supported by the cell proliferation in the basal part of the leaf lamina. Thus, a phyllomorph can be regarded as a leaf primordia with a prolonged leaf expansion. In *Arabidopsis thaliana*, we found that *bop1* mutant does not cease the cell proliferation in the basal part of the leaf lamina. *BOP1* homolog of *Monophyllaea* may be a key to understand the genetic basis of the phyllomorph.

6.5.5. Did giants evolve from dwarfs? Developmental morphology of *Gunnera herteri*.

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The genus *Gunnera*, mainly distributed in the Southern Hemisphere, includes herbs varying in size, from a few centimetres to several metres. Recently, phylogenetic studies based on molecular and morphological data highlighted the relationship among the species. One of the best-supported results regarded the position of *Gunnera herteri* at the base of the tree as sister to the rest of the genus. While the other species of *Gunnera* are rhizomatous or stoloniferous perennials, *G. herteri* is a tiny annual, differing in these and many other traits from the other species of *Gunnera*. Rare and with a restricted distribution in Uruguay and Brazil, *G. herteri* has been seldom collected and its developmental and flower morphology have been poorly known. Here, we summarize our studies on *G. herteri* and discuss whether the peculiarities of this plant are autapomorphies connected to the annual habit, or if the ancestral species of *Gunnera* were actually dwarfs that developed into giants.

6.5.6. Saltational loss of root-shoot bauplan in *Dalzellia zeylanica* (Podostemaceae) with crustose vegetative shoot, compared to closely related *Indotristicha ramosissima* with roots and shoots

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The Podostemaceae are aquatic rheophytes with remarkably specialized morphology. Saltational evolution amplifies the diversity of their morphology as seen in species pairs with distinctly different morphologies. *Dalzellia zeylanica* lacks a root while its shoot is foliose and adheres to rock surfaces. A unique complex meristem at the shoot margin is involved in the coenosomic shoot structure. By contrast, *Indotristicha ramosissima*, sister to *D. zeylanica*, has subcylindrical roots and root-borne, branched shoots. Seedlings of *D. zeylanica* have no obvious primary shoot

and no root; one of two shoots formed in the axils of two cotyledons grows into a secondary shoot. Seedlings of *I. ramosissima* have secondary roots arising from the hypocotyl. The saltational evolution of the *D. zeylanica* bauplan may be due to drastic early ontogenetic changes, such as appearance of secondary shoots in the epicotylar region and loss of the root, as well to modifications, such as flattening and adherence of the shoot compensating functionally for loss of the root.

6.5.7. Concluding statements: Classical plant morphology in the context of dynamic morphology*

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Classical plant morphology and dynamic morphology represent two theoretical frameworks used to describe and understand the bauplan of vascular plants, especially flowering plants. Generally, plants are interpreted in terms of three mutually exclusive structural categories: stem, leaf, and root. Stem and leaf together constitute a shoot which usually shows axillary branching. According to the classical approach, organ identity can be predicted by its relative position within the plant's bauplan. This model applies to many but not all flowering plants. There are groups with forms that do not clearly fit into the classical model. In these cases, a dynamic morphological perspective may serve as a more encompassing model. It accepts developmental mosaics between stem, leaf, and root. This continuum model was revived during the pre 'EVO-DEVO' period by Agnes Arber, Rolf Sattler, and others. Some of the ideas of this dynamic approach are compatible with results obtained by evolutionary developmental plant biologists. * dynamic morphology = continuum morphology

6.6.1. Late Paleocene floras of North Dakota, USA: New sites and new insights

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A recently recognized Late Paleocene flora at Beicegal Creek, western North Dakota, USA provides new information about floristic elements known from the classic Almont flora. Whereas the original Almont site yields silicified shale that shows a combination of external morphology and some of the only known anatomical detail for Paleocene plants, the new site offers permineralized remains more similar to the silicified "bog" floras of the Eocene Princeton chert and Miocene Yakima Canyon. In contrast to the surface occurrence at Almont, fossils from Beicegal Creek are found in place and can provide details about depositional environment and stratigraphic relationships. Even outcrops in close vicinity to one another show heterogeneity in both floristic composition and preservation. Newly recognized floristic elements occur at this locality, including ferns and an aquatic monocot, and some plants currently known from Almont occur in greater numbers e.g. (*Spiromatospermum*, *Musaceae*). The higher level of resolution also allows for greater taxonomic comparison in currently known taxa (e.g., *Amersinia*, *Cornaceae*; *Palaemyrtinaea*, *Myrtaceae*).

6.6.2. Fossil woods from the Paleogene of Russian far east

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Additional information about plant biodiversity in Russian Far East (RFE) during the Paleogene has been obtained due to the study of fossil woods collected from several sites in Kamchatka, Sakhalin, the Lesser Kuril Islands, Primory'e and Khabarovsk Territory. As a result of the study 30 species assigned to genera: *Podocarpoxyylon* (*Podocarpaceae*), *Abietoxyylon*, *Cedus*, *Keteleerioxylon*, *Laricioxyylon*, *Piceoxyylon*, *Pinuxylon* (*Pinaceae*), *Metasequoia*, *Sequoioxylon* (*Taxodiaceae*), *Cupressinoxylon* (*Cupressaceae*), *Alnus* (*Betulaceae*), *Engelhardioxylon* (*Juglandaceae*) were identified; among them 19 species have been described as new ones. Fossil woods studied show mostly a mixing of anatomical features of the extant species growing in Siberia, RFE, Korea, China, Japan or western North America. Fossil woods of the *Engelhardioideae* and *Cedrus* were found in Russia for the first time, *Alnus* and *Keteleeria* - for the first time in RFE. The Sikhotealin has been proposed to consider as a second center of species formation in the *Larix* during the Late Oligocene. The work

was supported by the Presidium of Russian Academy of Sciences (grants 05-1-P12-022, 05-1-P25-078).

6.6.3. Origin of early Paleogene deciduous flora in the Pacific Sector of Arctic

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The late Maastrichtian age of the Koryak Flora (NE Russia) is established based on correlation of plant-bearing and marine deposits. The Sagwon Flora (North Alaska) is dated as the Paleocene by palynological data. The Koryak Flora has much in common with the Sagwon Flora. Dominant components in both floras are dicots and conifers. The Sagwon Flora of a lower taxonomic diversity includes almost all taxa known in the Koryak Flora. Absence of characteristic Cretaceous taxa indicates a younger age of the Sagwon Flora. The close similarity of these two floras evidences against a hypothesis of a global catastrophic event at the Cretaceous-Paleogene boundary. Development of floras was likely controlled here by climatic fluctuations, evolution and migration of plants. Presumable consequences of meteoritic impacts are recorded in the middle-latitude North America only and have not been global in scale. Floristically and physiognomically the Koryak flora has much in common with Early Tertiary floras of North-eastern Asia and North America. Probably, it was the Koryak flora that gave rise to the Early Tertiary deciduous floras of the Arctic and adjacent regions.

6.6.4. The phylogeographic history of Rhizophoraceae*

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The fossils of 4 genera of Rhizophoraceae, *Rhizophora*, *Kandelia*, *Ceriops* and *Bruguiera*, distributed in the strata of Tertiary to Quaternary in Asia, Europe, Africa, Oceania and America. The fossil record of Rhizophoraceae indicated (1) Rhizophoraceae was highly probable originated in the Tethys seacoast from Paleocene through Early Eocene, and rapidly dispersed to Asia, Europe, Africa, Oceania and America during Middle Eocene; (2) during Oligocene Rhizophoraceae continued to spread in Asia, Europe, Africa, Oceania and America, but it became extinct in Europe since Oligocene; (3) during Miocene Rhizophoraceae became a characteristic taxon across tropical-subtropical seacoast of Asia, Africa, Oceania and America; (4) from Pliocene through Pleistocene Rhizophoraceae reduced markedly, and then began to flourish in Holocene. The distribution pattern and phylogeographic evolution of Rhizophoraceae distribution closely correlated to the continental drift, Quaternary glaciation and global climates changed.

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6.6.5. A soboliferous acaulescent palm from the deccan intertrappean beds of India

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A permineralized matured soboliferous acaulescent palm from the Deccan Intertrappean bed exposed at Umaria, District Mandla, M.P., India has been reported. The specimen exhibits a large tuberous mother plant having stem covered with sheathing leaf bases, some of them enclosing axillary peduncles. The young plant arises monopodially. Some of the leaf bases of the young plant also enclose peduncles in their axils. The stem apex is crowned with spirally arranged young leaves. The stem of the mother plant and also the stem of the young plant exhibit vascular bundles typical to Palmae. The inflorescence axis is woody, elongated in cross section at the base and becoming circular towards the distal part is covered with the spathes. Anatomically it shows a compact arrangement of fibrovascular bundles along with trace, diminutive and fusion bundles. The spathe covering the peduncle shows parallel arrangement of the vascular bundles in the mesophyll tissue. The morpho-anatomical characters suggest the affinity with the soboliferous acaulescent palms probably belonging to subfamily Coryphoideae and tribe Phoeniceae.

6.6.6. Novel applications of confocal laser scanning microscopy: examining fossil plants

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Confocal laser scanning microscopy (CLSM) has been in common use for about 20 years. However, the application of confocal microscopy in paleontological research has been largely limited to microfossils and mammal dentistry. This study presents some of the first uses of confocal microscopy for research on plant fossils. CLSM allows for the excitation of autofluorescent plant tissues and compounds such as phenolics. Some lauraceous material from the Middle Eocene Princeton Chert has previously been noted for its autofluorescent characteristics. We demonstrate the utility of confocal techniques in examining permineralized fossil fruits, seeds, pollen, and vegetative organs. In particular structures with complex secondary compounds are prime candidates for this technique. Use of the CLSM results in dramatically increased image resolution, and also allows for the production of three dimensional reconstructions using computer software.

6.7.1. Introduction: A perspective on species radiation - the New Zealand story

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This talk will provide a perspective on the contribution that studies on the New Zealand flora are making to understanding the phenomenon of species radiation. As such it will act as an introduction to subsequent talks of the symposium. It will also consider the problems that species radiation pose for phylogenetic reconstruction. One aim of the talk is to explain the motivation for some newer methods of phylogenetic analysis that may facilitate biological interpretation of plant species relationships. Methods such as neighbor-net, consensus networks, split decomposition and supernetworks are expected to be most helpful when species relationships are non-bifurcating, and when gene trees are incongruent, due to hybridisation, lineage sorting and/or phylogenetic error. To illustrate how different features and properties of sequence data may be identified and distinguished using phylogenetic networks, my presentation will include observations on angiosperm radiation, as well as observations on the radiation of alpine plants in New Zealand.

6.7.2. New Zealand as a model system for investigating plant biodiversity: a comparison with volcanic island archipelagos.

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Oceanic islands have long served as the paradigm for studying evolutionary processes such as species radiations. These model systems provide outstanding opportunities to study such microevolutionary processes due to their extreme isolation and comparatively straightforward geological and geographic histories. Islands that are vestiges of Gondwana, such as New Zealand, with highly complex yet well understood geographical and geological histories can also offer much to the study of evolution. In this paper I will describe the geological and climatic features unique to each of these systems and how they act to promote species radiations. Examples from the floras of the Hawaiian Archipelago and New Zealand will illustrate how the physical characteristics of these diverse systems have shaped the plant biodiversity. I will conclude with a discussion of how the study of New Zealand's plant biodiversity can further our understanding of plant evolution particularly within insular systems.

6.7.3. Significance of long-distance dispersal in the evolution of the New Zealand flora

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The New Zealand archipelago has a unique prehistory - originally part of the Gondwanan supercontinent, New Zealand has been isolated and oceanic in character for much of the last 80 million years. Furthermore, since the mid to late Tertiary tectonism,

volcanism, eustatic sea-level change, and glaciation have dramatically modified the landscape. This highly dynamic prehistory has profoundly influenced the composition of the contemporary flora. New Zealand's extensive fossil record documents the extinction of many plant groups and suggests that many plant lineages have only arrived since its isolation from the other Southern Hemisphere landmasses. Recently, molecular phylogenies and age estimates have provided important new insights. We review molecular evidence for the importance of long-distance dispersal in shaping the modern New Zealand flora. More specifically that progenitors of many plant groups have arrived by long-distance dispersal since the mid Tertiary. We also describe examples in which New Zealand plants have colonized habitats in Australia, New Guinea, and South America.

6.7.4. Morphological diversity of New Zealand plants: species radiation and convergence on an oceanic island group

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A distinctive feature of the New Zealand oceanic island flora is species radiation into a variety of forms and habitats, for which examples are presented. An increasing number of these radiations, including those of gnaphalioid Asteraceae, Brachyglottis, Ranunculus, and Myosotis, have been shown to have very little genetic divergence compared with their morphological and ecological differentiation. This contrast implies rapid species radiation, which is consistent with the extensive geological and climatic changes in New Zealand in the Pliocene and Pleistocene. There is a link between these explosive radiations and other distinctive features of the New Zealand flora such as divaricating habit, heteroblasty, white or inconspicuous flowers, cushion habit and mobile scree plants, because radiated species groups often contribute members to these convergences. One result of this is that similar forms often belong to a range of unrelated families. We present the example of plants of mobile screes.

6.7.5. Hybridisation and polyploidy in the New Zealand angiosperm flora

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Hybridisation is often advanced as an explanation for taxonomic difficulty in New Zealand angiosperm groups, especially where extensive hybridisation occurs in cultivation, e.g., *Veronica*. Until recently there has been little empirical support for extensive introgression, although some interspecific and intergeneric F1 hybrids have been well documented. Several recent studies have demonstrated polyploid hybrid speciation (e.g., *Ranunculus*, *Thelymitra*). However, the possible role of diploid hybrid speciation has not yet been widely examined. Many large genera have speciated at one level of ploidy (often hexaploid) in New Zealand, but some (e.g., *Leptinella*, *Veronica*) show extensive polyploidy and aneuploidy. Poor phylogenetic signals from ITS sequence data have been assumed to indicate rapid and recent diversification in the flora. However in some cases incongruence of cpDNA and ITS gene trees might be evidence of extensive hybridisation over a longer time period. I will present some New Zealand examples that use a novel analytical approach based on gene tree incongruence for the recognition of hybridisation in a phylogenetic context.

6.7.6. Plant mating strategies and their role in adaptive radiation

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Plant mating strategies govern genetic transmission across generations and can both drive and limit species radiations. While mating systems such as dioecy are associated with lower species number, others such as self-fertilisation can enforce reproductive isolation. The New Zealand flora is characterised by radially symmetric, white flowers pollinated by an unspecialised insect fauna. Moreover, greater than 10% of the native species are dioecious and over 20% are predominantly selfing. To investigate the link between mating systems and species radiation in New Zealand I used published phylogenies to compare species richness and breeding system of monophyletic genera or sub-

genera in New Zealand with their overseas sister taxa. Most New Zealand taxa are less, or no more, speciose than their sister group. However, there are a few cases of striking species radiations in New Zealand (e.g. *Veronica*, *Myosotis*, and *Aciphylla*). Most New Zealand groups examined had smaller, less specialised flowers than their sister taxa, irrespective of their species richness. In New Zealand breeding system may limit, rather than drive, species radiations.

6.7.7. *Pachycladon* (Brassicaceae) as a model plant in the study of species radiation

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Model plants are a powerful tool to understand evolutionary processes and to investigate the genetics underlying morphological and ecological diversity. The New Zealand *Pachycladon* are a monophyletic group closely related to the model plant *Arabidopsis thaliana*. They are similar to this species in terms of sequence divergence and chromosome synteny. Species of *Pachycladon* differ in growth habit, leaf and rosette morphology, the number of times they flower, and seed morphology. There are nine *Pachycladon* species indigenous to the Southern Alps of New Zealand and these are considered to represent a recent and rapid radiation. The geological and climatic framework in which *Pachycladon* has evolved is well understood, providing the opportunity to investigate genetic and also environmental drivers of species radiation. Utilising the genetic and molecular resources developed for *Arabidopsis*, we report on the potential of *Pachycladon* as a model plant for investigating the genetics underlying morphological and ecological diversification during species radiation.

6.8.1. Phylogeny and evolution of Rosaceae

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Rosaceae are systematically challenging from subfamilial to species levels. Molecular data strongly support as monophyletic the two largest traditional subfamilies, Maloideae and Rosoideae, although with somewhat altered composition. Neither of the other two traditional subfamilies, Amygdaloideae and Spiraeoideae, is monophyletic, and instead their genera plus some cast-offs from Rosoideae fall into about eight lineages, each with a few genera. Molecular analyses have so far failed to resolve fully and strongly relationships among these clades, hinting at the possibility of a rapid radiation of the family early in its history. Most of these clades are heterogeneous for carpel and fruit characters, suggesting complex patterns of morphological evolution. Hybridization has been inferred in the ancestry of the Maloideae and within and among numerous genera of Rosaceae. The interplay of hybridization, polyploidy, and agamospermy has created numerous agamic complexes in the Maloideae and Rosoideae. Intricate evolutionary relationships in these complexes have made consistent application of any species concept extremely difficult.

6.8.2. Rosaceae in Neotropical montane forests and paramos

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The family Rosaceae is economically and ecologically important in the Neotropical region, several native species are used for wood, food, and also as medicinal plants; however, there are relatively few studies of the Rosaceae in this region. In the Neotropics occur 17 genera of Rosaceae with about 220 native species, 50% of these genera are endemic from Central and South America, such as *Hesperomeles*, *Lachemilla* and *Polylepis*. Other genera probably migrated from the northern hemisphere such as *Prunus* and *Rubus*, although those have several native Neotropical species. The Rosaceae in the Neotropics are mainly distributed at high elevations in the montane forests and páramos, between 2000 and 5000 m. *Lachemilla* is one of the most diverse genera of Rosaceae in the Neotropics with approximately 80 species

distributed in the western Neotropical mountains, about 60% of the species are found from Venezuela to Bolivia, above 3000 m, which suggests that the elevation of the Andes was important for the evolution and diversification of this genus. First molecular analyses have confirmed a close relationship of *Lachemilla* to *Alchemilla* and *Aphanes*.

6.8.3. Major clades in the evolution of Rosoideae

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Recent phylogenetic analyses of Rosoideae based on chloroplasts and the nuclear DNA sequence data mostly agree on six well supported clades: Filipendula (10 spp.), Rubus (c. 700 spp.), Colurieae (c. 70 spp.), Sanguisorbeae (c. 275 spp.), Rosa (c. 100 spp.), and Potentilleae (500+ spp.). The three latter comprise the Roperculina clade. As delimited since the landmark contribution by Morgan et al. (1994), Filipendula is sister to the remainder of Rosoideae. Separate analyses of chloroplast and nuclear data have shown different relationships among the remaining clades, particularly in the position of Rubus. Ribosomal ITS data favour Rubus + Colurieae, while noncoding chloroplast data favour Rubus + Roperculina, but not in all cases. Analyses of GBSSI (waxy) exon data do not show a consistent pattern with current sampling, when the two loci present in Rosaceae are compared. Analyses of the sequence data using model-based approaches may shed light on these relationships, as well as the position of Rosoideae within Rosaceae.

6.8.4. Evolution of Maloideae with a focus on *Crataegus*

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The last 10 years have seen progress not only in deducing generic relationships within Rosaceae subfamily Maloideae, but also in obtaining novel evidence for relationships between species in some of the larger genera. *Malus* and *Pyrus* have been the subject of studies aimed at discovering relationships between wild and cultivated genotypes. Data from molecular markers have also corroborated earlier inferences from cytology and morphology about the origins of *Sorbus* hybrids. In *Crataegus* a major breakthrough has been the ability to determine ploidy level by means of flow cytometry. This makes it possible to concentrate on diploid taxa initially, and to predict the number of gene copies to be expected when adding triploids and tetraploids to the sample. Molecular data support the view that morphological variation in *Crataegus* encompasses that seen in *Mespilus*. Other genera such as *Amelanchier* and *Aronia* have been studied up to now only in North America. *Cotoneaster* remains as a major challenge for future work.

6.8.5. Molecular Evolution in the Rosaceae.

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During the past decade molecular systematic studies have refined our understanding of relationships within Rosaceae and helped resolve a long-standing evolutionary question. Our first insight into Rosaceae molecular systematics came from analyses of *rbcl* sequences by Morgan et al. (1994) who demonstrated that chromosome number was more representative of relationships than fruit type, and Maloideae may have originated from Spiraeoid progenitors. The first study of nuclear DNA sequence variation used *nrlts* (Campbell et al. 1995) and identified a close affinity between traditional Spiraeoid genera and Maloideae as well as small clades within Maloideae. Both were jumping off points for numerous studies, with the most recent using sequence from the multi-copy, nuclear-encoded GBSSI gene. The presence of multiple copies of GBSSI genes has provided a powerful tool for recent molecular studies (Evans et al. 2000, Evans and Campbell 2002, Smedmark et al. 2003). Collectively these analyses have

used multiple copies of GBSSI to demonstrate the importance of hybridization in *Rosoideae* genera, as well as the North American origin of *Maloideae*.

6.8.6. Evolution of gametophytic self-incompatibility in the Rosaceae

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In the Rosaceae, the control of self-incompatibility is gametophytic, which means that the rejection reaction is conditioned by the genotype of the male gametophyte. RNases are the stilar product of the gametophytic self-incompatibility locus in three families: the Rosaceae, Scrophulariaceae, and Solanaceae. Most studies on the evolution of RNase-based self-incompatibility undertaken so far have dealt with the Solanaceae and have revealed extensive sequence divergence and transspecific evolution (closely related alleles are found in different species or even genera). Both extensive sequence divergence and transspecific evolution result from the negative frequency-dependent selection acting on the locus. The Rosaceae, however, have shown some striking differences compared to the Solanaceae. In particular, sequence divergence is much lower and, although extensive transspecific evolution has been observed within subfamilies *Maloideae* and *Amygdaloideae*, no overlap has been observed between those subfamilies. Various equilibrium and nonequilibrium scenarios may be considered to account for the observed differences.

6.8.7. Hybridization as an evolutionary factor in *Rosoideae* with a focus on *Rosa*.

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Hybridization is recognized as a major mode of evolution in plants. However, the distribution and frequency of this evolutionary factor is uneven among plants. Within Rosaceae, subfamily *Rosoideae* is subject to intensive reticulation processes. One of the most remarkable genera within *Rosoideae* with respect to systematic biology is the genus *Rosa*. The realization of multiple reproductive strategies from apogamy to reticulation, matroclinal character inheritance, auto- and allopolyploidy, concert between environmental dependent and independent speciation and intensive networked genome evolution in the genus limit concepts of diversification, radiation, speciation and subsequent taxonomy. An analysis of the evolutionary patterns and processes in *Rosa* gives on one hand clear evidence for the stochastic character of this specific evolutionary scenario. On the other hand certain results are comparable to evolutionary strategies in other polymorphic taxa and shed light on general patterns and processes of reticulate evolution in polyploid plants.

6.9.1. Aspects of fern radiations

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Leptosporangiate ferns exist as a separate lineage at least since the early Carboniferous. They underwent several major diversification and extinction events that shaped their extant diversity. Recently, it was shown that the most diverse lineage of leptosporangiate ferns, the polypods, diversified not earlier than the Cretaceous. Thus, at least 80% of extant fern diversity evolved after the appearance of the angiosperms. In this presentation, major questions concerning the global pattern of fern radiations are addressed with the focus on events in the last 100 million years. Putative factors triggering radiations of ferns are discussed based on selected lineages such as asplenoid and polygrammoid ferns. Various factors are considered such as climatic change, colonization of new habitats, and morphological/physiological key-innovations. Several radiations respectively diversifications of fern lineages are explored using phylogenetic evidence with and without estimation of divergence times. Finally, current evidence about patterns of fern radiations are compared with theories concerning the process of diversification or radiation.

6.9.2. Dating the diversification of pleurocarpous mosses

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Molecular phylogenetic analysis of pleurocarpous mosses shows the presence of very short internal branches in the base of the pleurocarpous "bush", indicating the possibility that they diversified rapidly over a (geologically) short period of time. However, the absence of a good fossil record of mosses in general, and pleurocarpous mosses in particular, has hindered attempts to determine when this group evolved and the processes implicated. In particular, the idea that the evolution of pleurocarpous mosses was associated with the appearance of angiosperm forests has been untestable. Here we explore the patterns of diversification of the pleurocarpous mosses and other bryophytes using sequence divergence based methods and estimate possible dates for their origins. The appearance of pleurocarpy was dated at 161-194 mya, but the majority of pleurocarp lineages appear about 131-165 mya. This latter event potentially coincides with the origin of angiosperms in the Early Cretaceous, but clearly predates the appearance of complex angiosperm forests in the early Cenozoic. The hypothesis that pleurocarpous mosses evolved to exploit the angiosperm forests needs to be modified - instead, it is suggested that pleurocarpous mosses diversified in parallel with the early angiosperms, contributing to the development of the complex modern ecosystems.

6.9.3. Fast and slow filmy ferns: molecular rate heterogeneity a chloroplast-wide phenomenon in the Hymenophyllaceae.

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The Hymenophyllaceae is the largest family of basal leptosporangiate ferns, comprising more than 650 species in two large sister genera (*Trichomanes* and *Hymenophyllum*). Although these two genera are equally rich in species number, they are quite different in terms of ecological diversity; *Trichomanes* exhibits five ecological modes (terrestrial, climbing, hemi-epiphytic, lianescent, and epiphytic), whereas *Hymenophyllum* is exclusively epiphytic. Recent *rbcl* studies have revealed striking differences in the rate of molecular evolution between *Trichomanes* and *Hymenophyllum*, with *Hymenophyllum* showing a significant rate deceleration. Here, using a taxonomic sampling of 25 species for each genus, we show that this same pattern is present in two additional regions of the plastid genome, and is therefore likely a chloroplast-wide phenomenon. Molecular divergence time estimates also confirm that *Hymenophyllum* diversified much more recently than *Trichomanes*. Possible explanations for the observed molecular rate differences are examined in the context of what is known about the ecology and life history patterns of these ferns.

6.9.4. Diversification of heterosporous ferns: Kovach and Batten revisited

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Heterosporous ferns (Salviniales) are the most recent evolutionary experiment with heterospory and are one of many taxa, along with angiosperms and polypodiaceous ferns, that transformed terrestrial ecosystems during the Cretaceous and Paleogene. Extant families Salviniaceae and Marsileaceae form a monophyletic clade, but are morphologically distinct from each other, limiting our ability to test hypotheses about morphological transformations. However, heterosporous ferns and their relatives (inferred from megaspores) possess an extensive fossil record from the Cretaceous through to the Recent. Our morphological phylogenetic analysis including fossil and extant taxa supports the inclusion of *Balmeisporites* and *Ghoshispora* in crown group Salviniales, and previous conclusions about a marsileaceous affinity for *Arcellitites* and inclusion of *Glomerisporites* and *Parazolla* in Salviniaceae. By combining new discoveries and phylogenetically-based spore affinities with an expanded data base of occurrences, we update the work of Kovach and Batten (1993) who first described quantitatively heterosporous fern diversification.

6.9.5. The diversification of epiphytic ferns: evidence for a radiation on angiosperms

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The epiphytic habit has clearly been of paramount importance in the evolutionary history of ferns, with epiphytes accounting for more than 30% of extant fern species. Nonetheless, remarkably little is known about the diversification of these plants, and critical questions concerning the timing and patterns of epiphyte evolution remain unanswered. Recent analyses of fossil and molecular data have revealed that the bulk of extant fern diversity arose subsequent to the rise of angiosperms in the Cretaceous, perhaps as an opportunistic response to the development of more complex angiosperm-dominated habitats. The latest Cretaceous and earliest Tertiary establishment of multistratal, angiosperm-dominated, everwet tropical forests provides a reasonable stimulus for the diversification of epiphytic ferns. Here, this hypothesis is evaluated by estimating the timing and patterns of diversification for both epiphytic and terrestrial fern lineages, within a broad phylogenetic framework.

6.9.6. Diversification of Hawaiian Ferns

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The native Hawaiian fern flora is composed of approximately 140 species, classified in 67 genera. We and several of our colleagues have conducted evolutionary studies of various groups of Hawaiian ferns. The combined results across groups are helping us discern several patterns of speciation and diversification. 1) Multiple/repeated colonizations of single, widespread species that have not undergone speciation post-colonization; e.g. *Asplenium adiantum-nigrum* and possibly such species as *Sphenomeris chinensis*, *Dicranopteris linearis*, and others. 2) Small radiations of species in relatively specialized habitats (e.g. epiphytes in wet forest), presumably from similar specialized species/colonizers; e.g. Grammitidaceae. 3) Small radiations of species with apparent more generalist ecological adaptation from ecologically similar, but relatively narrowly distributed non-Hawaiian colonizers; e.g. some of the groups of *Dryopteris*. 4) Single endemics diverging from widespread species but not radiating into more species rich endemic groups; e.g. *Asplenium hobdyi* and *Dryopteris subbipinnata*.

6.9.7. Exploring the radiation of asplenioid ferns using phylogenetic and population genetic approaches

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Phylogenetic studies of *Asplenium* on a global scale have allowed us to date historic radiations in this species rich genus. We are comparing these results with population genetic and biosystematic investigations into European taxa in order to infer general patterns and processes of evolution and radiation.

With more than 50 taxa *Asplenium* is the most species rich fern genus in Europe. Climate fluctuation and habitat disturbance during Pleistocene glaciation cycles has led to range contractions and expansions in many plant species. Contact and hybrid zones were formed, resulting in repeated polyploid formation. Minority cytotype exclusion prevented the mixing of the different cytotypes and supported the establishment of distinct cytotype ranges. We use the rock fern genus *Asplenium* to explore the dynamics of speciation and extinction in relation to environmental change.

6.10.1. The origin and early diversification of eudicots

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Eudicots comprise the bulk of extant angiosperm species, and much of the broad phylogenetic structure within the group has now been clarified through the application of phylogenetic techniques

based on molecular sequence data. In the fossil record eudicots are first recognised in rocks slightly younger than those containing the earliest evidence of angiosperms. In the first palynofloras and mesofloras in which eudicots can be recognised they are subservient to putative monocots and magnoliids (*sensu lato*) in both diversity and abundance. Subsequently, they quickly become important and early fossils include forms related to extant *Platanus*, Buxaceae and perhaps Trochodendraceae. Eudicots then diversify rapidly through the mid-Cretaceous. There is further rapid diversification through the late Cretaceous and into the Cainozoic. A steadily increasing number of extant lineages can be recognised through the Cretaceous and early Cainozoic in a pattern that is broadly consistent with that predicted by phylogenetic analyses of extant taxa.

6.10.2. Basal eudicots from the mid-Cretaceous of Euroasia

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Fossil plant assemblages comprising reproductive structures, pollen and leaves have been studied in Central Europe, Kazakhstan and Western Siberia. Two major groups of basal eudicots, platanoids and trochodendroids, have been recognized. Platanoids are represented by the staminate globular inflorescences, *Sarbaya*, which has tetramerous flowers, and *Platananthus*, which has pentamerous flowers. Both genera show massive stamens with peltate apical parts of connectives. Trochodendroids in Western Siberia are characterised by the reproductive structure, *Nordenskiöldia*, and leaves of *Zizyphoides* type. In Central Europe, they are represented by unequivocal pollen grains. Eudicots in Euroasia are further represented by the intriguing pistillate reproductive structure, *Cathiaria*. It consists of a main axis with helically arranged, flattened lateral axes bearing adaxially unilocular sessile fruits. The associated staminate racemose inflorescence, *Freyantha*, is characterized by bracteate flowers bearing tricolpate, reticulate pollen. In all known Euroasian localities *Cathiaria* co-occurs with the compound biternate leaves *Liriodendropsis*.

6.10.3. The Cretaceous and early tertiary history of primitive eudicots in North America

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Eudicots migrated from the tropics to North America by early Aptian. Representatives include tricolpate pollen-characteristic of all eudicots-and *Vitiphyllum*, an herbaceous plant with leaf architecture suggestive of Ranunculales. By the late Albian at least three of the four major clades of primitive eudicots were present. Proteales were a major component of the angiosperm record; they include *Nelumbites*, a primitive relative of Nelumbonaceae, and the *Sapindopsis*/platanoid complex, which has features that link it to Platanaceae and Proteaceae. During the latest Albian and Cenomanian platanoids underwent a major adaptive radiation. During the Late Cretaceous Platanaceae and Trochodendrales/Cercidiphyllales were an important component of high-latitude deciduous vegetation, while Platanaceae and Menispermaceae were minor components of lower-latitude evergreen vegetation. During the Paleocene Platanaceae and Trochodendrales were common components of mid-latitude deciduous forests. During the Eocene modern genera of Menispermaceae were a diverse component of thermophilic rainforests, which were widespread at middle northern latitudes.

6.10.4. The timing of eudicot early diversification

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The onset of eudicot diversification gave rise to a number of lineages with distinct morphologies, some of which may represent the baseline condition for characters possibly associated with the diversification of core eudicots. We investigate the initial phases of eudicot diversification to address the timing of origin of major early eudicot lineages and the age of their crown groups, the diversification dynamics of species-poor and diverse clades, and the rates of molecular evolution associated with the origin of early eudicot lineages. Dates and molecular rates were estimated by applying penalized likelihood to a parametric hypothesis of

phylogeny constrained with ages derived from fossils. Our results suggest the rapid differentiation of major eudicot lineages soon after the origin of eudicots, and, in many cases, a substantial temporal gap until the origin of their crown groups. Distinct species diversification patterns seem to characterize differentially diverse early eudicot clades: whereas some lineages are presently depauperate survivors of formerly diverse clades, at least one species-rich clade resulted from a recent rapid radiation.

6.10.5. Biogeography of the early eudicots

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The early eudicots, consisting of the Ranunculales and several other lineages (e.g., Proteales, Buxales, Sabiales), contain taxa with geographic distributions throughout the world. Various vicariant/dispersal hypotheses are proposed to explain the patterns of distribution found within this diverse group, concentrating on those taxa with distributions in the Southern Hemisphere. In the Ranunculales, geographic patterns found in some groups (e.g., *Anemone* and *Caltha*, Ranunculaceae) strongly suggest a former Gondwanan distribution. Other families (e.g., Menispermaceae) may have a Gondwanan origin but, due to more recent dispersal events, the pattern is less evident. In the non-ranunculalean lower eudicots, several lineages (e.g., Proteaceae) also suggest a Gondwanan distribution. To test the roles of vicariance and dispersal in selected taxa, the following lines of evidence will be explored: 1) paleogeographic reconstructions using parsimony-based tree fitting to identify concordant geographic patterns, 2) the fossil evidence, and 3) the congruence of the dates for various geologic events with the latest time-calibrated lower eudicot trees.

6.10.6. Floral structure and evolution in Proteales and Buxales

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Buxales (Buxaceae, Didymelaceae) and Proteales (Nelumbonaceae, Platanaceae, Proteaceae) belong to the partly unresolved grade of early diverging eudicots situated between Ranunculales and core eudicots. Early diverging eudicots are characterized by a high evolutionary plasticity in floral organ differentiation and flower organization. This is in contrast to the rather fixed conditions within the core eudicots. With the exception of Proteaceae and Nelumbonaceae, perianth organs are often more or less bract-like and undifferentiated. Floral organs are arranged in whorls in all members of Buxales and Proteales, except for Nelumbonaceae and female flowers of Buxaceae, which both have spiral phyllotaxis. Within a whorl, organs may either be initiated simultaneously or successively. Most often are the floral organs organized in a dimerous decussate pattern. Trimerism and pentamerism are occasionally found in the flowers of fossil and extant Platanaceae, and the uppermost perianth organs of female flowers in Buxaceae tend towards a pentamerous arrangement. Developmental studies of extant Platanaceae indicate a high variability of floral organ merosity.

6.10.7. Patterns of MADS-box gene duplication and the evolution of the ABC program in the lower eudicots.

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The elucidation of the genetic program controlling floral organ identity has created opportunities for understanding the evolution of floral morphology. In particular, it appears that aspects of floral diversity could have been generated through simple shifts in the functional domains of the homeotic organ identity genes. Members of the lower eudicot family Ranunculaceae are well suited for testing this hypothesis due to their wide array of perianth types. In many genera, two whorls of morphologically distinct petals are present in the flower, indicating that two separate petal identity programs may be functioning. The genus *Aquilegia* is very useful in the study of this phenomenon due to its small diploid genome and the availability of many floral homeotic mutants. We have identified homologs of all the major Arabidopsis floral organ identity genes in *Aquilegia*, and have begun to characterize their expression patterns and protein interactions. It appears that gene duplications which occurred in the APETALA3 lineage before the diversification of the Ranunculaceae have facilitated the evolution of multiple forms of petaloid organs in this new model species.

6.11.1. Evolution of the Compositae: the big picture.

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One of every 10 flowering plant species is in the Compositae. Although monophyletic, there is much diversity among the members. Ideas about classification and diversification remained largely unchanged from Bentham through Cronquist. The results of molecular studies were used to produce a meta-supertree formed by the grafting of the tribal trees onto a base supermatrix tree. Examination of the distribution of terminal taxa indicates that the origin of extant members of the Compositae was in southern South America. A subsequent radiation in Africa gave rise to most extant tribes. The African radiation contains clades from Asia, Eurasia and Australia. There was a North American origin and diversification of the Heliantheae s. l. The pattern might suggest a Gondwanan origin for the family but data indicate a more recent origin. A monotypic North American genus is between the South American and African radiations. The sister-group to the western hemisphere clade Heliantheae s. l. is a small tribe from tropical Africa. A global picture of the Compositae provides a framework for studies in morphology, evolution, and diversification and highlights areas in need of work.

6.11.2. Phylogeny and biogeography of Barnadesioideae, the most ancient subfamily

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Barnadesioideae, with 91 species in nine genera, represent the most ancient lineage of Compositae. This small complex is confined to South America, with a concentration in the Southern Cone and with extensions along the Andes north into Colombia. Morphologically the group contains much variation, ranging from small herbs (such as in *Doniophyton*) to trees 20 m tall (in *Dasyphyllum*). Recent cladistic analyses based on separate morphological and molecular data have shown different patterns of intergeneric relationships. New analyses of selected corolla characters support patterns based on molecular data, in which *Barnadesia* and *Huarpea* are ancestral within the group (as opposed to *Schlechtendalia*, hypothesized previously). Implications for early biogeography of the subfamily are explored, with data suggesting origin and diversification in southern South America followed by subsequent radiation southward into Patagonia and also northward into the Andean chain. This second lineage, accompanied by selection for hummingbird pollination, resulted in a wide range of different morphological forms.

6.11.3. Molecular phylogeny of Cardueae: the complete picture

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The classification of Compositae has changed dramatically in recent years. The new outline of relationships in basal branches confirms that the sister group to the large tribe Cardueae are not Mutisieae, but only a small group of African genera of this tribe that are now classified as tribe Tarchonantheae. This change implies that our outgroup selection in previous molecular studies was not suitable, and monophyly of Cardueae must be reassessed on a molecular basis. Moreover, new collections in recent years allow us to extend our sampling to 70 of the 74 genera of the tribe. We performed a new molecular study of the tribe using one nuclear region (ITS) and two chloroplastic ones (*trnL-trnF* and *matK*) and a more appropriate outgroup. Our results confirm that Cardueae are a natural group but indicate some changes in subtribal delineation: a new subtribe Cardopatiinae is recognized and some genera are moved to other subtribes (*Myopordon*, *Nikitinia*, *Syreitschikovia* and the *Xeranthemum* group). A recapitulation of a number of interesting questions that remain unresolved in the classification of some large genera is presented.

6.11.4. Phylogeny and subtribal delimitation of the *Cichorieae* (Asteraceae)

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Subtribal delimitations within the Asteraceae-Cichorieae (c. 100 genera and over 1550 species) are often controversially discussed as delimiting morphological features are scarce. Analyzing DNA sequences of ITS and *matK* of a broad representation of the family and combining molecular and morphological data, we discovered robust support for 10 monophyletic subtribes: (1) Scolymiinae including *Catananche*, (2) Scorzonerinae, (3) Cichoriinae comprising *Cichorium*, *Tolpis* and *Arnoseris*, (4) a primarily North American clade comprising the Microseridinae, Malacothricinae, and Steophanomeridinae, (5) Hieraciinae sensu stricto, (6) Lactucinae sensu stricto, (7) Chondrillinae including *Urospermum* and *Phitosia*, (8) Crepidinae including *Nabalus* and *Rhagadiolus*, (9) Sonchinae including *Hyoseris* and *Dendroseris*, (10) Hypochaeridinae sensu stricto but including *Prenanthes* sensu typo. Our phylogeny confirms an origin of the tribe in Eurasia. General history and distribution of the tribe are discussed.

6.11.5. Small tribes, but a large challenge: clades and grades of Arctotideae and Liabaeae

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The primarily Andean Liabaeae with c. 180 spp. contains two monophyletic and one paraphyletic subtribes. Within this opposite-rosulate-trinerved-leaved clade, the fate of its largest, paraphyletic genus *Munnozia* of the Munnoziinae, is of key interest. The systematic position of the southern African genera *Hoplophyllum*, *Eremothamnus*, *Heterolepis*, and *Platycarpha* remains uncertain, although they most likely are closely related to the Arctotideae (with c. 220 spp.), distributed in the same phytogeographical area. Arctotoid disc floret styles is the only putative morphological synapomorphy for this tribe and the molecular support is rather weak. In contrast, both subtribes are diagnosed by morphological synapomorphies, such as anthers with an obsolete filament collar, radial endothecium, and soft apical appendage (Arctotidinae), and connate phyllaries, 4-lobed rays, and sclerified disc floret lobes (Gorteriinae). In the Arctotidinae, Arctotis and *Haplocarpha* are paraphyletic, but a large portion of species of Arctotis form a clade. A parallel situation is found in the Gorteriinae, where both *Hirpicium* and *Berkheya* are paraphyletic.

6.11.6. Progress in understanding Vernoniaeae evolution

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The combined *trnL*-*rbcL* + *ndhF* + ITS results of Keeley, Forsman and Chan (2004) are compared with taxonomic, geographic, chemical, palynological, and other aspects of the Vernoniaeae. *Vernonia* s.s. is mostly North American taxa. Recent concepts of the paleotropical *Gymnanthemum*, and the Neotropical *Critoniopsis* seem too broad. Paleotropical ancestry of the tribe is confirmed. The yellow-flowered, trinerved-leaved *Distephanus* is basal. *Centrapalinae* and typical *Gymnantheminae* are near basal. The paleotropical *Erlangiinae* I and II are most advanced, group I with mostly sublophate Type A pollen and 5-methyl-coumarins, group II with Type A and triporate pollen and eremanolides. Neotropical taxa have all pollen types, sublophate often obviously derived. *Elephantopinae*, with dilactones, and *Manyonia* reached the paleotropics with some evolved differences. Paleotropical *Centratherum* and *Struchium* were introduced by humans. Some paleotropical taxa relate to the *Eremosis* group. More limited *trnL* DNA show strictly Neotropical relationships for *Acilepidopsis* with separate origin of triporate pollen.

6.11.7. An updated outline of the tribe Mutisieae s.l. (Asteraceae)

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The tribe Mutisieae s. l. (Asteraceae), with 85 genera and ca. 960, species is extremely diverse morphologically. Its basal position enhances the tribe's value as a key for understanding the systematics, evolution, and biogeography of the whole family. Since the elevation of Barnadesiinae to subfamily status, the Mutisieae is constituted by 3 subtribes: Mutisiinae, Gochnatinae, and Nassauviinae. The first two were joined in Mutisiinae s. l. Nassauviinae is monophyletic but Mutisiinae is taxonomically complex, constituted by generic groups, isolated genera, and taxa of dubious position. Molecular phylogenies exclude *Ainsliaea* and relatives, *Dicoma* and relatives, *Gochnatia* and relatives, *Brachylaena* and *Tarconanthus*, *Hesperomannia*, *Oldenburgia*, and *Hecastocleis* from Mutisieae s. str. but a final resolution of the tribe taxonomy has not been achieved. The last morphological studies of Mutisieae s. l. agree with the exclusion of many of these taxa, but not with the exclusion of *Gochnatia* and relatives, *Ainsliaea* and relatives, *Oldenburgia*, and *Hecastocleis*. The value of the style and pollen morphology in the classification of Mutisieae is emphasized.

6.12.1. Soil seed banks in Europe: What do we know?

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Even in Northern Europe, little is known about seed banks of species that occur outside the farmed landscape, or about rare species in general. Seed bank data are also of extremely variable quality, and the seed bank of an individual species cannot be confidently classified on the basis of fewer than five independent records. This picture is changing only slowly. Most new data concern species whose behaviour is already well-known. Data for new species occur only rarely and it will be a very long time before we have good empirical data for most European species. Prediction of persistence from seed traits has been only moderately successful. Physiological seed dormancy and persistence in soil are scarcely related. Seed size and shape are related to persistence in the soil, but the relationship cannot predict the behaviour of individual species. Defence against pathogens is linked to long-term persistence in the soil, but the variety of defence chemicals involved, and the difficulties of analysis, mean that this will never be a practical predictor of persistence. Here I review research on European seed banks and reflect on the likely future progress of this research.

6.12.2. Long-term investigation of seed dispersion on a sandy grassland in a Kiskunsági National Park

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We have analysed the species diversity in seed rain investigations in the mosaic complex of the grass conozis that were taken over 20 years. The dominant species of the vegetation were *Festuca vaginata*, *Festuca pseudovina*, *Plantago indica*, *Crepis capillata*, *Silene otites*, *Dactylis glomerata*, *Koeleria glauca*, *Poa angustifolia*, *Chrysopogon gryllus*, *Secale silvestre*, *Crepis rhoeadifolia*, *Silene longiflora*, *Dianthus serotinus*, *Stellaria graminea*, *Poa bulbosa*, *Calamagrostis epigeios*, *Molinia coerulea*, *Cynodon dactylon*, *Medicago minima*, *Seseli osseum*, *Polygonum arenarium*, *Prunella laciniata*. We have classified the seeds that were taken from 60 places based on similarities in quality and quantity. The factors that affected the groups were irrigation, isolation, chemical fertilization and the removal of the plant species. The temporary forms that represent the secondary succession stages of the plant communities can be determined based on the seed samples.

6.12.3. Common, but not forgotten: population biology of *Trachymene incisa* (Apiaceae)

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Population studies frequently focus on threatened species and overlook the common but ecologically interesting plants that make up the diversity of the Australian bush. Our studies of *Trachymene incisa* have revealed that this herbaceous perennial plant has a complex life cycle which includes seeds, rosette plants, and dormant adult tap-roots. These components of the population ensure that *T. incisa* is capable of multiple responses to disturbances such as fire. Plants may either resprout from a tap root or seeds may germinate from a seed bank. Interestingly, there are two forms of seed bank in these populations; a transient aerial

seed bank and a long-lived soil seed bank. These complex life history attributes are incorporated into a stage-based matrix population model. This enables us to evaluate the relative contributions of the two seed banks and the adult dormancy to the population growth rate. The demography of two populations of *T. incisa*, one of which had recently been burnt, are compared and the consequences of the seed bank for population persistence are presented.

6.12.4. Seed germination strategies investigated using computer simulations.

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In the Sonoran Desert environment of the Phoenix area, rainfall is irregular and unpredictable in quantity and frequency. Some plants survive dry periods by existing as seeds during unfavorable periods. Approximately one half of the native plant biodiversity near Phoenix consists of these ephemeral plants that exist mainly as seeds in a seed bank. A desert ephemeral whose seeds all germinated on first exposure to moisture might quickly become extinct. A better strategy might be to germinate only a fraction of its seeds in a particular growing season, thus hedging the risk. A computer simulation program has been developed that models various climates and plant characteristics to suggest strategies that might be effective under different conditions. The results of these simulation experiments indicate that the best strategies for ephemeral plants in a Sonoran desert environment seem to match some of the observed germination characteristics of actual desert plants, e.g. *Pectocarya recurvata* (Boraginaceae).

6.12.5. Germination and establishment of snowbed plant species of the North-Eastern Calcareous Alps

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The template of suitable habitats will shift upwards for most alpine species due to climate change. Area loss and fragmentation will especially concern species restricted to small and well-defined habitat patches such as snowbeds. Germination and successful establishment of seedlings are assumed to be rare in alpine regions but data on germination and establishment rates are still lacking for most alpine species. This study focuses on ten selected snowbed species of the North-Eastern Calcareous Alps, Austria. Germination experiments with different stratification treatments were carried out in a climate chamber. The seedlings of the four most successfully germinating species were transplanted to a snowbed on Mt. Rax and monitored throughout one vegetation period. Germination rates for these latter species ranged from 75 to 93 %. Among treatments stratification with gibberellic acid shortened time to germination but enhanced seedling mortality. Mortality of transplanted seedlings was generally low (5 - 14 %). We conclude that a considerable part of alpine plants may successfully recruit from seeds at surprisingly high rates under favourable conditions.

6.12.6. Genetic structure of experimental populations and reproductive fitness in a heterocarpic plant *Atriplex tatarica*

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Atriplex tatarica L. is a heterocarpic species of man-made habitats with a mixed mating system and wide geographic distribution. Seeds of *Atriplex tatarica* do not germinate immediately after shedding, but may remain in a dormant but viable state indefinitely. We investigated the influence of genotype and fruit type of *Atriplex tatarica* on germination and establishment rates. We asked whether there were genetic and fitness differences between plants derived from seeds of the different fruit types that germinate (a) early versus late, and (b) under different ecological conditions (temperature and salinity). We found that the allozyme allele frequencies varied significantly with both fruit type and germination time under temperature and salinity treatments. When single locus heterozygosity was taken into account, we found significant differences only for germination time under temperature treatment and for fruit type under salinity treatment. Moreover, fitness components, i.e. the amount of generative and vegetative

biomass, of particular plants within experimental populations increased with their increasing heterozygosity.

6.13.1. Mosses and alternative adaptation to life on land

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Bryophytes represent optimal adaptation to land life at a scale two orders of magnitude smaller than vascular plants. At this scale surface tension is a major force, gravity is trivial, and bryophytes often lie largely or wholly within the laminar boundary layer of the air; the contrast is comparable to that between insect and mammalian physiology. Most of the cell physiology of bryophytes is essentially the same as vascular plants, but water storage and movement are largely external, and mosses generally deal with intermittent availability of water by tolerance of desiccation rather than by regulating water loss. Photosynthesis by moss shoots tends to be limited by CO₂ diffusion at high irradiance (when photoprotection becomes important); few show light-saturation levels above 1000 μmol m⁻²s⁻¹ PAR. This diffusion limitation is escaped by Polytrichales and Marchantiales which have evolved ventilated photosynthetic systems analogous to vascular-plant leaves; these structures relate to enhancing CO₂ uptake rather than conserving water.

6.13.2. Desiccation tolerance in the liverwort *Jamesoniella colorata*.

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The leafy liverwort *Jamesoniella colorata*, has two distinct colour morphs; red-brown and green. In higher plants the functional significance of these (red) accessory pigments has been ascribed to reducing cell damage. One of these proposed protective roles involves desiccation tolerance. The susceptibility of *J. colorata* to desiccation and subsequent rehydration was studied.

When gametophytes were desiccated chlorophyll fluorescence parameters only changed at the onset of turgor loss. After rewetting, the recovery of photosynthesis was more complete in the red gametophytes than in the green. The results suggest that non-radiative energy dissipation is an important factor in recovery from desiccation. Changes in stress indicators, such as increase in lipid peroxidation and decrease in ascorbate content, could only be detected after rehydration. Chlorophylls and carotenoids only decreased upon remoistening the samples in low light. The data indicate that accessory pigmentation shields the photosynthetic apparatus from excessive radiation at vulnerable stages and thereby enhances tolerance to adverse climatic conditions.

6.13.3. An integrated model of bryophyte canopy water and carbon dynamics.

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In bryophytes, canopy structure controls light attenuation thereby affecting photosynthetic rates and also the duration of photosynthetic activity by governing water loss. Canopies may not optimize light capture and water retention simultaneously, leading to potential physiological tradeoffs. To explore these tradeoffs, a two-component model of canopy carbon and water exchange was constructed. For the photosynthesis component, the canopy was stratified into 5 mm layers. For each layer, shoot area, light and photosynthetic light response curves were combined to calculate carbon exchange rates. Light attenuation and shoot area were modeled as a function of surface roughness (L_r), a structural parameter. The water exchange component employed a boundary-layer model that relates conductance to L_r . Increased L_r led to greater light penetration and higher whole-plant photosynthetic rates. In contrast, increased L_r caused greater rates of water loss, reducing photosynthetically active time. The model predicts that bryophytes occupying mesic habitats would benefit from high L_r , whereas under xeric conditions, low L_r should enhance carbon gain.

6.13.4. Acclimation to within-canopy light gradient in four moss species of contrasting architecture

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Although bryophytes typically grow in deep shade, they seldom grow as individual shoots, but form tightly packed cushions with strong vertical light gradients. We studied light acclimation of photosynthetic activity along the moss canopy light gradients in branching (*Pleurozium schreberi*, *Scleropodium purum*) and non-branching (*Polytrichum commune*, *Tortula ruralis*) species to test the hypotheses that light gradients strongly modify moss physiological activity and that light gradients are stronger in branching mosses. We studied the rate of photosynthetic electron transport (J_{max}), nitrogen (N_M) and chlorophyll (Chl_M) and estimated moss LAI and light interception in one cm intervals along the canopies. Mosses intercepted 95-99% of incident irradiance, implying extensive light gradients in the canopies. Branching mosses intercepted more light than non-branching species. N_M decreased from the top to the bottom of plant canopies by 1.5-3-fold, while Chl_M decreased by 5-20-fold. These changes were accompanied by 2-3-fold changes in J_{max} , demonstrating major light-dependent physiological modifications.

6.13.5. Effects of an altitude-dependent natural gradient of UV-B radiation on the physiology of sun and shade populations of an aquatic liverwort

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We report the effects of a 676-m altitudinal gradient of UV-B radiation on the physiology of the aquatic liverwort *Jungermannia exsertifolia* subsp. *cordifolia* coming from mountain streams in northern Spain. Photosynthetic pigment composition, photosynthesis and respiration rates, some variables of chlorophyll fluorescence, UV-absorbing compounds (UVAC), protein concentration and sclerophylly were measured in 11 sun populations and 7 shade ones. In sun plants, UVAC levels, ETR_{max} and NPQ_{max} increased with increasing altitude, whereas photoinhibition percentage decreased. These characteristics may confer tolerance to high UV-B levels. The concrete UVACs whose concentration increased with increasing altitude were chemically identified. Shade plants did not show such congruent response to altitude, probably because the UV-B gradient was scarce for these plants. A Principal Components Analysis summarized the influence of both altitude and precedence of the samples (sun vs. shade) on the physiology of *J. cordifolia*. Globally, this liverwort showed highly plastic physiological features in response to UV-B radiation.

6.13.6. Seasonal variations in storage substances and sensitivity to predators in the moss *Grimmia pulvinata*

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Mosses are generally resistant to herbivory, with some exceptions that often include young sporophytes. Although they produce substances of nutritional value, some secondary metabolites have been suggested to play a deterrent role. In *Grimmia pulvinata*, we have observed predation, mainly by slugs, affecting only immature capsules. In this research, the content of storage substances (sugars, starch and lipids) has been analysed during a complete annual cycle. Qualitative observations have been obtained using histochemical techniques. Enzymatic detection and MS spectroscopy were performed for quantitative analysis. These substances are subjected to seasonal variations. The lipidic fraction includes mainly fatty acids and aliphatic alcohols, together with biologically active compounds such as vitamin E, sterols, and triterpenes. Starch and sugars are detected especially in structures growing actively. Among them, the gametophyte tissues and capsules after sporogenesis show a high lipidic content, probably making them resistant to predators. In turn, young capsules, with a reduced lipidic fraction, would remain unprotected.

6.14.1. From source to shelf or an introduction to the supply chain of medicinal and aromatic plants

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At least every fourth flowering plant is a medicinal and aromatic plant (MAP) in use. The high demand in MAP for domestic and commercial use results in a huge trade from local to international level. Not less than 400,000 t worth US \$ 1.2 billion are traded internationally each year, unknown are those volumes produced

and consumed within the countries. The international trade is dominated by only few Asian and European countries. Until now, the production of botanicals relies to a large degree on wild-collection, which is not necessarily detrimental in itself, but, the increasing commercial collection, largely unmonitored trade, and habitat loss have led to an unsustainable pressure on plant populations in the wild. World-wide an estimated 4,000 MAP are threatened. Possible ways to address the threat to the future supply of MAP and their habitats range from resource management, cultivation, shifting value-adding in source countries, species conservation to trade restrictions. As the majority of MAP will continue to come from wild-collection in the foreseeable future, MAP are of high conservation priority to secure their supply for local healthcare and trade.

6.14.2. Sustainable wild collection of medicinal and aromatic plants: Development of an international standard

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Between 40,000 and 50,000 plant species are known to be used in traditional and modern medicine systems throughout the world. Uncounted additional species are used in the growing cosmetics and botanicals industries. The great majority of these materials is provided by collection from wild resources. Notwithstanding growing interest in cultivation, wild collection is likely to continue as the principal source for most species of medicinal and aromatic plants, in most parts of the world, based on biological, economic, and social factors. Building on successes in the development of sustainable harvest and resource stewardship standards in other sectors such as timber, non-wood forest products, organic agriculture, and marine aquarium fish, an initiative has recently been launched to develop an international standard and criteria for good practice in the sustainable wild collection of medicinal and aromatic plants. This paper summarizes the current status of work to develop this standard, supported by the German Federal Agency for Nature Conservation, WWF/TRAFFIC Germany, IUCN Canada, and the IUCN Medicinal Plant Specialist Group.

6.14.3. The role of associations in sustainable sourcing - perspectives from collectors and processors

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The paper presents the views of Southern African producers and processors (all of whom are members of PhytoTrade Africa - The Southern African Natural Products Trade Association) on sustainable sourcing of MAP. It notes that producers accept the need for independent verification of sustainability, but are concerned that certification procedures carry realistic transaction costs and do not become barriers to trade. The paper emphasizes the need for contextually appropriate indicators of sustainability, and highlights the steps that can, and cannot, be taken by associations to promote efficiency in the adoption of certification systems. Examples are given of how existing certification systems actually inhibit moves towards sustainability, tend to favour wealthy organised commercial farmers over wild-harvesting rural poor, and the practical steps that PhytoTrade Africa has taken to promote sustainable production amongst its membership. The paper concludes by urging pragmatism in the development of standards and criteria for sustainable sourcing.

6.14.4. Collectors training: basis for trade promotion

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SIPPO (Swiss Import Promotion Programme) supports private businesses in emerging markets and markets in transition that are endeavouring to access the Swiss and European market. In Bosnia Herzegovina SIPPO promotes companies which collect medicinal and aromatic plants in a sustainable way. SIPPO therefore trains companies and collectors and has developed together with an organic certifier a manual for good collection practices and monographs with practical information for the most collected plants in this area. This training and the documentation coming out of the certification helps companies to sell their products in the European market and gives them the possibility to trace their products back to the area of collection and the collector.

This is a niche market in the commerce of MAPs and the agents and retailers are prepared to pay a higher price for these added value products. Important in this setting is the constant training and checking of the collectors in order to ensure the sustainability of medicinal and aromatic plants.

6.14.5. Bio trade: implementation of conservation and sustainable use practices along the value chain

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The BioTrade Initiative of the United Nations Conference for Trade and Development (UNCTAD) supports developing countries to promote trade in products and services derived from biodiversity with the aim of contributing to sustainable development. BioTrade programmes in Latin America, together with national and international partners from private and public sector, have been providing support to enterprises and their providers along the whole value chain. The latter need to comply with an increasing number of compulsory or voluntary guidelines that assure sustainability of their sourcing practices. Assistance on conservation and sustainable use practices is therefore provided, taking into account market requirements, local legislation, voluntary guidelines and BioTrade principles and criteria. The implementation of such practices involves the development of protocols and training programmes, which are elaborated together with enterprises in each country. Experience shows the importance of promoting integrated approaches that support different actors along the whole value chain, involving the private sector and providing market incentives.

6.14.6. Certification of wild products - an important tool for sustainable crop management

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Exploitation of natural flora has become a major threat to biodiversity in many regions. While collection of wild plants is for millions of people the sole source of income, a global market drains the resources and promotes unnecessary product loss by low quality and deterioration. Certification is based on standards. The set up of standards for sustainable collection practises is the starting point of a certification system. Such standards should contain as many relevant criteria as possible, but to start with simple standards is better than not to start at all; standards can and should always be improved with increasing knowledge of the specific situation of the ecosystem. Standards are also an important tool to train the collectors as in many regions collecting is done by immigrants with no traditional knowledge of ecology and the ecosystem. In addition, post-harvest handling should be included in the standards and need to be monitored and verified by the inspection procedures in order to increase the percentage of high quality output. And - of course - certification goes hand in hand with a traceability system which is a precondition for a global market approach.

6.15.1. Soil community feedbacks and plant community dynamics

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While the direct effects of soil microorganisms on plant growth are known to be profound, evaluation of the importance of soil organisms on plant community processes has been technically difficult. I outline an approach to evaluating the role of soil organisms in plant community dynamics that involves testing the feedback on plant growth through host-induced changes in the composition of the soil community. I use this approach to test the importance of soil organisms in the coexistence of competing grasses and forbs and generally find that the composition of the soil community changes in a manner that decreases the growth rate of their host species relative to that of a second plant species. These negative feedbacks on host growth result from accumulation of host-specific soil pathogens, and from shifts in the composition of mutualistic arbuscular mycorrhizal fungi and rhizosphere bacteria. The negative feedbacks can allow coexistence of strong competitors, with the long-term dynamics being inferred from general community models.

6.15.2. Root secondary metabolites: pure chemical defence or negative feedback to facilitate soil microfungi coexistence

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An important function that is attributed to the so-called secondary plant metabolites is chemical defence that guarantees survival in the ecosystem in contrast to primary metabolites that are essential for life sustenance. However, recent developments of hypotheses that focus biotic interactions, and especially such occurring belowground, as important factors structuring plant communities, challenge this view especially in regarding negative feedbacks as favourable for species coexistence. Bottom up regulated microbes are well known to produce antibiotic secondary metabolites that have shown to contribute to the coexistence of strains in a way resembling the computer game rock-paper-scissor. I propose to extend this view to secondary metabolites which are often accumulated in huge amounts in plant roots and present a major constraint to microbial decomposers, the majority of them soil fungi showing high species and strain diversity.

6.15.3. Plant-soil feedbacks and exotic invasions

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Evidence is increasing for the important role of soil biota in exotic invasions. Invaders may escape inhibitory biota, encounter facilitative biota, or alter soil microbial processes in invaded ecosystems. We find that plant-soil feedback processes shed light on exotic invasions in both Europe and North America. *Centaurea maculosa*, a European invader of North America, and *Prunus serotina*, an American invader of Europe, participate in different plant-soil feedback processes at home compared with their home ranges. In native soils, these invaders cultivate soil biota with increasingly negative effects on their growth, possibly leading to their control. But in soils from invaded ranges, *Centaurea* and *Prunus* cultivate soil biota with increasingly positive effects on themselves, which may contribute to the dramatic increase in ecological success that these species experience in their new ranges. We attribute these biogeographic differences to pathogen-plant relationships that are more host-specific than mutualist-plant relationships. In sum, the feedback loops that develop between soil biota and an invasive plant depend on the biogeographical source of the microbes.

6.15.4. Shrub facilitation and feedback with soil fungi contribute to succession in a chaparral-oak forest Mediterranean ecosystem

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Quercus ilex L., the holm oak, is widely distributed around the Mediterranean basin. In mature forests of Corsica, recruitment is very low in despite significant acorn production. However, this evergreen species establishes well in chaparrals dominated by *Arbutus unedo* L. (the Strawberry tree). We investigated whether microbe-mediated interactions are driving forces in the chaparral-forest transition. Acorns were introduced in plots protected against animal predation, and their growth and survival rates were monitored during one year. Plant-soil feedbacks were investigated through a manipulation of microbial communities using (i) a reciprocal soil transfer between the chaparral and the oak forest and (ii) a soil fumigation approach. We also examined the ectomycorrhizal colonization of the seedlings, and we identified the fungal symbionts using molecular methods. We found greater survivorship and ectomycorrhizal colonization of oak seedlings beneath the canopies of *A. unedo* shrubs, (ii) soil-related effects on establishment of seedlings, and (iii) a significant effect of the fungicide on seedling survival in forest soils.

6.15.5. Response of an arid woodland to N fertilization: contrasting ecto- and arbuscular mycorrhizal conifers

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Nitrogen deposition is escalating from urbanization and agriculture in arid regions. I applied 100kg N/y in a pinyon pine-juniper woodland in New Mexico, USA from 1997 to 2004. Mixed EM Pinyon and AM juniper woodlands cover 20million ha. Soil NO₃- and NH₄⁺ increased with fertilization but soil C declined. Isotope data indicate that turnover is increasing in soils beneath pinyon but stable C is increasing under juniper. Root length, and % and total mycorrhizae did not change, although both varied with changing rainfall. Based on ¹⁵N of EM sporocarps and pinyon, 60 to 80% of leaf N was provided by EM fungi, likely as organic N. Juniper appeared to use soil inorganic N. Leaf %N increased in pinyon (2%) but decreased 11% in juniper. ¹⁵N increased by 0.80/oo in pinyon but only 0.30/oo in juniper. Leaf production increased in 50% in juniper, and 33% in pinyon. However, pinyon began to suffer from mortality from drought in the fertilized plots (40% versus none in the controls), whereas juniper thrived. These data indicate that EM conifers may be especially sensitive to N deposition whereas AM conifers may be adapted to the higher inorganic soil N.

6.15.6. Symbiont diversity as a driver of plant diversity and ecosystem functioning

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Symbiotic interactions between plants and micro-organisms are widespread and very abundant in nature. About 150,000 plant species associate with arbuscular mycorrhizal fungi (AMF) and more than 15,000 species of legumes form intimate relationships with nitrogen fixing rhizobia bacteria. In this talk, evidence will be presented showing that both AMF and rhizobia play a key role in ecosystems. These microbes contribute to plant diversity and ecosystem productivity by stimulating growth and nutrition of their host plants. AMF and rhizobia also can act as support systems for seedling establishment in nutrient poor grassland. Interestingly, many legumes form tripartite symbiotic associations with both rhizobia and AMF, and several legumes appear to rely on both symbionts to flourish in natural communities. The fact that specific AMF communities are found to be associated with the roots of nitrogen fixing legumes suggests that synergistic interactions may exist of which previously has not been thought.

6.15.7. Plant-soil feedback: from black box to multitrophic interactions

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Plant-soil feedback has become a powerful concept for understanding influences of communities of soil organisms on plant community and vegetation development. However, in order to really understand consequences of feedback effects between plants and soil organisms for the performance of individual plant species and the result for vegetation development, it is necessary to further explore interactions below ground. Here I will propose how to apply the concept of multitrophic interactions in improving our understanding of plant community processes as influenced by feedback effects from the soil community. I will use plant defence approaches developed for aboveground interactions between plants, herbivores and carnivores and show how different plant defence strategies may result in succession, plant species diversity and plant invasions. Then, I will show that individual plant species may exhibit more than one defence strategy at the same time using results of the EU-EcoTrain project on multitrophic interactions between plants, root-feeding nematodes and natural antagonists in the root zone of a natural dune grass (*Marrham grass; Ammophila arenaria*).

6.16.1. Vascular plants as indicators of overall species richness

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The selection of sites for management of biodiversity is a difficult task. A detailed inventory of all species is practically impossible. Therefore, one is forced to use some form of surrogates or indicators of overall species richness. Basic requirements for a successful use of vascular plants as a surrogate species group are discussed. A selection strategy based on species richness hotspots requires that hotspots for vascular plants are hotspots for the target species groups as well. Whereas a selection strategy

based on complementarity requires that target species groups respond to the same major environmental gradients as vascular plants. It is concluded that vascular plants may be a useful indicator species group, especially in a complementary site selection. In addition to common gradients with several other species groups, vascular plants are well-known, they are easily identified in the field, and their gradient relationships (vegetation types) are well-studied.

6.16.2. The relationship between landscape disturbance and biodiversity using ecological indicators and a site comparison index

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The relationship between habitat disturbance and biodiversity has strong implications for both ecological theory and land conservation strategies. Research to identify ecological indicators of landscape disturbance was conducted in the complex Fall-Line Sandhills physiographic ecotone of southeastern USA (Fort Benning, Georgia). Forty sites were selected representing the full range of military training disturbance and upland vegetation communities. Seven ecological indicators were analytically identified, standardized, and weighed by statistical criteria to develop a composite Site Comparison Index (SCI). SCI transect scores were grouped into five ordinal disturbance classes. Within this disturbance gradient, 33 metrics of biological diversity were statistically evaluated (16 for ground cover, 9 for trees, and 8 for ants). Diversity metrics included: species richness, abundance, dominance, and Simpson, Shannon, Brillouin diversity and evenness indices. The effect of disturbance on biodiversity varied dramatically with the metric employed. A number of patterns substantiated the intermediate disturbance hypothesis.

6.16.3. Searching for indicators of epiphytic crustose lichen diversity: Macrolichens, forest variables and climatic variables

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Species richness is a fundamental measure of biodiversity, and global trends of declining species richness are a major ecological concern. However, gathering data on species richness is expensive and time-consuming. Consequently, cost-effective methods have to be developed. Our focus is on indicators for crustose lichen diversity. Crustose lichens are a very species rich group, but they are laborious to sample and identify and, thus, are often neglected. In a large scale study, we found significant relationships between species richness of crustose lichens and the easily assessable macrolichens (R²: 0.38 - 0.70, depending on substrate group). Currently, we are searching for better models for epiphytic crustose lichens by adding different sets of variables (forest stand variables and climatic conditions) to the epiphytic macrolichen data. If easy-to-measure forest stand variables sufficiently improve the models, epiphytic crustose lichen richness could cost-efficiently be predicted. Moreover, for designing efficient forest reserve networks, we are studying the complementarity between both epiphytic macrolichens and crustose lichens.

6.16.4. An approach for the identification of indicators for biodiversity

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The identification of indicators for biodiversity is of essential importance with regard to science as well as practical purposes. In the presented study, we focus on forest biodiversity and analyze published vegetation data of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), and European beech (*Fagus sylvatica*) forests on acidic soils in the lowland and mountain ranges of Germany. For different plant groups, such as bryophytes or Red List species, we investigate species numbers as a parameter of biodiversity. Species numbers are differentiated into three classes

to describe low to high diversity. Our approach focuses on the probability of being in a defined range of species numbers, i.e. class, if a certain indicator species occurs. Only few indicators can be found for the low ranges of species numbers. In addition, there are only a few species groups and stand types having indicators for all three classes. Various species have multiple indicator functions, e.g. with regard to the investigated species groups. The catalogue of indicators resulting from the investigation can help to facilitate and accelerate biodiversity evaluations of forest stands.

6.16.5. Besides plants, soil organisms provide added value as indicators for conservation and restoration success

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Conversion of arable land into semi-natural grassland or heath land is a major practice for restoring and conserving plant diversity, but little is known about the restoration of taxonomic and functional diversity in the soil. In a chronosequence of abandoned fields we determined how plant and soil communities develop along a secondary succession gradient. Plant community development proceeded according to a clear succession towards the theoretical plant associations *Galio hercynici-Festucetum ovinae* and *Genisto anglicae-callunetum*. However, succession of the nematode community was less well predictable. For nematodes, theoretical references are far less well developed than for plants, but similarity with a theoretical community indicative of arable land significantly declined with time since abandonment. Moreover, regarding similarity to natural reference sites, our results show that plant and soil nematode communities have individual trajectories of secondary succession after land abandonment. Therefore we argue that besides plants soil organisms provide added value as indicators for conservation and restoration success.

6.16.6. When two is better than one: a global analysis of complementarity patterns in flowering plant families

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An important focus of current conservation biology is the use of indicator taxa for rapid biodiversity assessments. However, an understanding of the efficacy of indicator taxa for biodiversity conservation is hampered by a lack of information on the distribution of biodiversity as a whole. Without this information, it is difficult to know whether patterns in the diversity of indicator taxa truly mirror such patterns in all of biodiversity. We used a unique database, developed and maintained at the Royal Botanic Gardens, Kew, which records presence or absence of all 14274 vascular plant genera across 52 major regions of the world to establish whether some plant families are more representative of global diversity patterns than are other families. Patterns of complementarity between large, widely-distributed families of flowering plants result in remarkably high correlations between global distributions of certain pairs or triplets of plant families and total genus-level flowering plant diversity across the regions studied. Conservation inventories focusing on these few families might therefore accurately reflect the total biodiversity of the region.

6.16.7. Life history characters and phylogeny are correlated with rarity in the Australian angiosperms.

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An exploration of four life-history traits (habit, life span, sexual system, fruit type) in the Australian flora (18 822 spp.) was undertaken to determine whether patterns can be extracted from our recently extinct and endangered species. Within the 31 extinct species we detected a significant departure from the expected values only for habit. There are significantly fewer trees on the extinct list than expected, reflecting perhaps the resilience of trees to extinction processes. Within the 450 endangered species we found significant differences within sex systems and fruit types. There are more monoecious species than expected by chance among the trees listed as endangered but fewer in the herbs and endangered herbaceous species are less likely to have dry-

indehiscent fruit types. A supertree analysis and character tracing showed that rarity was non-randomly clustered and occurs in some genera more often than expected by chance. This indicates that phylogeny is also an important component of rarity. We suggest that specific life history traits could be used in conservation planning and as an early warning sign for detecting vulnerability in lists of species.

6.17.1. Tropical ethnobotany

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Ethnobotany, the science of 'the botany of the people', explores the knowledge of people about plants - their types and how they are seen to be related, their uses and other properties, and how they live and can be managed. In principle, ethnobotany as carried out in the tropics is no different from ethnobotany carried out anywhere else, but the tropics tend to have certain features which make the subject especially apposite: the richness of the flora, a wealth of related folk knowledge of plants, the close dependency of many rural people on a diversity of local plants, both wild and cultivated, and the paucity of scientific knowledge of plants, giving added value to other traditions of botanical knowledge. Ethnobotany is emerging as a key subject for conservation and sustainable development in the tropics. This is leading to a more problem-centred and participatory approach to research, with local people and ethnobotanists working together to define the research questions, gather and analyse data, and explore the practical implications of the results. Applied ethnobotany is continuing to actively evolve. Some examples are given from the People and Plants Initiative.

6.17.2. Variation of traditional knowledge of the plant element among Zapotecs of three municipalities at the Sierra Madre del Sur, Oaxaca

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Traditional knowledge is the result of perception and interpretation of natural environment. Different expressions reflect the way native cultures develop traditional knowledge. One of these expressions corresponds to the folk nomenclature applied to natural elements like plants and animals. Zapotec group represents one of the most important ethnic group at the Sierra Madre del Sur, state of Oaxaca. As a consequence of thousand of years of interaction with local environment and, throughout a process of oral transmission, Zapotec people has developed a complicated folk system of the plant element. We interviewed 200 persons from three municipalities with different levels of aculturation, to know how many plants they know, Zapotec names, uses, where are they obtained and what part of the plant they use. We obtained 649 plant records, 10 plant communities and 11 plant life-forms. Levels of acculturation determined the variety of Zapotec names applied to plant life-form, vegetation types, number of plants species they recognized and uses they have. Keystone plant species were also those prominent species of the different ecosystems were people obtain plant species.

6.17.3. Naga home gardens & traditional knowledge of biodiversity conservation, Nagaland, India.

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Nagaland is one of north eastern states of India with wide range of cultural as well as biological diversity. Nagas have elaborated tradition of maintaining home gardens. These home gardens have been responsible for conserving valuable biodiversity. It is important to know the role of these traditional systems in conservation. In depth analysis of home gardens of Angami and Konyak Nagas and their relationship with higher biodiversity is presented in the paper.

Angami and Konyak Naga home gardens have many similar features as well as differences. It is attempted to define the linkages of these features and number of species that are cultivated and maintained through the indigenous knowledge of communities. Various functions of the home gardens, diversity within and among the home gardens, role of home gardens in domestication of wild species and development of varieties has been discussed in details.

Finally the role of home gardens research as a tool to develop methodologies for application of traditional knowledge in conservation and community development is discussed.

6.17.4. An ethnobotanical study in Wechiau community Hippopotamus Sanctuary in Ghana

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An ethnobotanical survey was conducted on the communities living within and round the Wechiau Community Hippopotamus Sanctuary in the Upper West Region of Ghana to understand and document the human uses of plants in the sanctuary. The study was conducted through direct interviews, questionnaires and personal observations. Seventy-six species of plants were identified as sources of food, medicines, fuel wood, animal feed, construction material, crafts and tools, as objects of culture and religion, and for other miscellaneous uses. In order of percentage use respectively, more plants were used for medicine (61.8 %), fuel wood (36.8 %), construction material (26.3 %) and crafts and tools (23.7 %). The sanctuary has therefore been identified as an excellent repository of Non-Timber Forest Products (NTFPs) whose conservation and sustainable uses will provide for the livelihood of the people in the locality.

6.17.5. Prioritizing conservation of medicinal plants in Sub-Saharan African forests

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The demand for traditional herbal medicine is increasing rapidly in Sub-Saharan countries mainly because of harmful effects of synthetic chemical drugs. However, the future of African medicinal plants lies on enhancing people's participation in the utilization and conservation of its forests. Worldwide a total of at least 35000 plants species are used for medicinal purposes. The subcontinent has a rich floral diversity, totalling about 24 000 species, with 4 000 species used in traditional medicine. Their use and conservation are across sectoral concerns that embrace not only the health care, but also nature conservation, biodiversity, economic assistance, trade and legal aspects. Today, unsustainable forest management is causing biodiversity loss and massive soil erosion, as well as negatively affecting the economic future of many communities around the globe, especially in the Sub-Saharan tropics. Programme activities need to be supported to reduce the rate of deforestation of the African tropical forests and conserve the biodiversity contained within them.

7.1.1. Transcriptional regulation of root hair development in Arabidopsis and its conservation among the land plants

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Root hairs are required for the uptake of minerals and water from soil. Hairs elongate by a mechanism known as tip growth, where cell expansion is focussed to a restricted region of the cell surface. Similar cell types are found throughout the land plants. A cascade of transcription factors regulates the development of root hairs in the model angiosperm Arabidopsis. Early acting transcription factors control the fate of cells in the root epidermis - epidermal cells may be hair-bearing or hairless. We have evidence that the early acting genes also control the expression of a suite of transcription factors that are required for late stages of root hair differentiation, when hair outgrowth occurs. One of these genes, *RHD6*, is only expressed in the root hair cell where it promotes the transcription of three other related genes which are also required for root hair cell differentiation. Evidence will be presented that defines the regulatory interactions between these genes in Arabidopsis and we will demonstrate that *RHD6* and *RHD6-LIKE* genes are part of an ancient mechanism that controls the differentiation of tip-growing cells in plants.

7.1.2. Stem cell specification and cell polarity in Arabidopsis roots

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Mutants in auxin transport and response indicate that distribution of this hormone is required for patterning distal stem cell

populations and control of cell division. Recent data suggest that transport-dependent auxin maxima are used not only in the root but also during embryogenesis and in shoot-derived organs. It becomes an important question how auxin as a patterning cue induces specific downstream pathways to mediate diverse effects. The *PLETHORA1* and *2* genes encode transcription factors required for stem cell specification and can ectopically induce root identity. *PLT* expression is auxin-inducible, depends on the auxin response factors MP and NPH4 and follows the basal auxin accumulation pattern during embryogenesis and in post-embryonic root development. *PLT* genes translate auxin accumulation into region- and cell type specification patterns, and interact with the SHR-SCR pathway that plays a role in patterning the root stem cells. The *PLT* genes regulate expression of the PIN facilitators of polar auxin transport and this contributes to a specific auxin transport route to maintain stem cells at the appropriate position within the root meristem.

7.1.3. Deciphering the transcriptional network for root radial patterning in Arabidopsis

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The simple symmetrical organization of the Arabidopsis root makes it an excellent system to analyze the molecular mechanisms regulating patterning during organ development. The root has a rotational symmetry along its longitudinal axis, generated through asymmetric cell divisions that take place in the root meristem. The transcription factor SHORT-ROOT (SHR) regulates the asymmetric cell division that generates the two sub-epidermal layers and is a key-component in the root radial patterning pathway. SHR was used as an entry point to reconstruct the transcriptional network controlling root radial patterning. We designed a genomic approach to identify the direct and secondary targets of SHR. We used a statistical analysis to combine information across 4 different microarray expression analyses, based on perturbation of SHR expression or activity. New insights into the mechanisms regulating root radial patterning will be discussed.

7.1.4. Genetic analysis of phloem development and differentiation in Arabidopsis

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We have shown that phloem development in the Arabidopsis root is established by a set of specific periclinal cell divisions. Resulting cell lineages differentiate into sieve element and companion cells. The recessive mutant *altered phloem development* (*apl*) lacks these divisions and the phloem-pole cells differentiate xylem characteristics instead of phloem (Bonke et al., 2003, Nature 426:181). *APL* encodes a MYB transcription factor active specifically in phloem cells. Ectopic *APL* expression results in inhibition of xylem development but not ectopic phloem development, indicating that *APL* is necessary but not sufficient for phloem differentiation. To identify additional factors in phloem development we performed a genetic screen of an EMS-mutagenized companion cell-specific marker line. This resulted in the identification of a set of novel mutants with patterning and/or cell proliferation defects specific to the stele. We present the characterization of these mutants. In combination with a forward genetic approach we aim to identify factors acting up and downstream of *APL* in the regulation of phloem development and differentiation in Arabidopsis.

7.1.5. Exploiting natural genetic variation in Arabidopsis to identify novel regulators of quantitative aspects of root development

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To isolate novel modulators of root growth, we exploited natural genetic variation in Arabidopsis. Two accessions with slow growing primary roots were investigated in detail. Through (back)crosses to control accessions, we Mendelized the major QTLs responsible for slow growth. So far, we isolated one of the two loci, named BREVIS RADIX (BRX), by positional cloning. This locus is responsible for ca. 80% of the variance of root length between the short-rooted and the control line, by controlling cell proliferation

and elongation in the root tip. BRX belongs to the highly conserved plant-specific BRX gene family. Analyses of multiple mutants suggest that BRX is the only gene of this family with a role in root growth. This lack of redundancy results from differential activity of BRX-like proteins as well as differential expression. BRX is nuclear localized, activates transcription in yeast and contains two highly conserved domains of unknown function. One domain mediates protein-protein interaction with another family of transcription factors. Thus the data indicate a role for BRX family proteins in transcriptional regulation. Further details will be reported.

7.1.6. Genetic analysis of root development in *Oryza sativa*

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Rice (*Oryza sativa*) root system at the seedling stages consists of a primary-, several crown- and many lateral roots. Although the importance of the root system for the maintenance of plant function has been well recognized, almost of the genetic and molecular biological bases of rice root morphogenesis remain still unknown. Recently genetic studies to verify the mechanisms of rice root development were started, and several root-type specific mutants were reported. However, the rice root mutants with a relevant tag that can be utilized for the isolation of the gene are still very rare. Therefore, we established a mutant screening system with *Ds*-tagged populations to dissect the root developmental mechanisms and to analyze the function of the genes in rice. As a result, mutant candidates showing root type-specific aberrations were selected. In this report, we introduce the phenotypes of the selected lines and discuss the implicated developmental mechanisms. This research was supported by a grant (CG1515) from Crop Functional Genomics Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology of Republic of Korea

7.2.1. Adaptive evolution of flowering responses to environmental cues in *Arabidopsis thaliana*

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The transition to flowering is regulated by diverse environmental cues such as photoperiod, vernalization, and ambient temperature, which mediate several different signaling pathways. Plants must integrate information from all of these pathways in order to flower at the appropriate time under dynamic real-world conditions. Both the environmental signals and the ecological factors that exert selection on the resulting phenotypes vary in space and time, so the optimal flowering response may vary geographically or across seasons. *Arabidopsis thaliana* is an ideal model system for investigating the functional and evolutionary significance of natural variation in the converging signaling pathways regulating reproductive timing. Ecotypes of *A. thaliana* from diverse climates exhibit considerable natural variation in developmental timing and its sensitivity to different environmental signals. Natural molecular polymorphisms at several key flowering time genes are associated with natural variation in developmental timing, as well as fitness under field conditions, and display geographic patterns suggesting adaptation to climate.

7.2.2. Chaperone Hsp90 as a molecular mechanism of genetic and environmental canalization

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The molecular chaperone Hsp90 plays a central role in the unfolding of genotype to phenotype. Manipulation of Hsp90 function yields a wide array of altered morphological phenotypes in plants and flies, which depend on underlying genetic variation. In *Arabidopsis*, manipulation of Hsp90 influenced phenotypic responses to environmental cues and buffered normal development from the destabilizing effects of stochastic processes. The multiple Hsp90 isoforms may be required to accomplish specific responses to the various external signals that plants must react to appropriately. To address the role of Hsp90 in the interplay of genotype and environment, we assembled a comprehensive set

of *Arabidopsis* lines reduced in different HSP90 isoforms via T-DNA insertion and RNAi approaches. We have used a combination of gene expression analysis, molecular and phenotypic assays to characterize novel phenotypes of adult Hsp90-reduced plant lines. These phenotypes suggest that Hsp90 plays an important role in organismic responses to biotic and abiotic environmental challenges and may facilitate the integration of signaling pathways, resulting in a canalized response.

7.2.3. A trade-off between root responses to waterlogging and nutrient patchiness - a general trend with *Rumex palustris* as an exception

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Roots are extremely responsive to changing soil environments, but it is yet unclear how responses to different soil factors are correlated. In a comparative study with 8 herbaceous species we examined whether selective root placement in nutrient hotspots and survival under water by adventitious root formation can be combined. Amongst 7 species there was a significant negative correlation among these responses, suggesting an ecological trade-off that may partly explain the distribution of the species in flooding gradients. One species (*Rumex palustris*) was an exception, with the highest propensity to explore enriched soil patches and the largest root system under water, indicating that the operation of these different root responses by a single plant is not physiologically constrained. Our results suggest that evolution has moulded the root responses of these species, depending on the specific dynamics of the soil environment.

7.2.4. Root to shoot signalling and the regulation of shoot growth and functioning in droughted plants

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Interactions between roots and drying soil generate chemical signals which move through the transpiration stream to shoots to regulate growth and functioning. Mild soil drying (and other edaphic variables) can modify the pH of the xylem sap and the shoot apoplast, thereby affecting the partitioning of the hormone abscisic acid (ABA) in the leaf to exert control on stomatal behaviour and growth. ABA-based control of these variables can be enhanced by sap alkalisation, even when the delivery of ABA to shoots is not enhanced by soil drying. Apoplastic pH can also be modified by climatic conditions such that this signalling catena can allow the plant to integrate edaphic and climatic influences such that stomata can respond appropriately to a range of environmental cues. More severe soil drying can influence the delivery of hormonal signals to the shoots and we highlight changes in the ABA and ethylene balance of shoots as soil drying intensifies. In this paper we show how each component of a signalling cascade from soil, through roots and stems to the leaves can influence the information content of the signal which arrives in the shoot.

7.2.5. The multifaceted implications of internal and environmental signals

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Plastic development necessitates that a plant coordinates its responses according to the detected conditions in the immediate environment. Recent evidence suggests that in many cases the very same mechanisms are responsible for mediating both the internal interactions between different tissues and organs of the same plant and the interactions of the plant with its external environment. Avoidance of inanimate obstacles by roots, self/nonself discrimination in roots and directional red/far-red sensitivity in shoots exemplify the multifaceted role of morphogenetical controls in plants. In all of these cases the very same physiological mechanisms are used to coordinate growth and development among different parts of the same individual plant and facilitate the perception and the adaptive responses to external stresses. It is suggested that the multifaceted role of some morphogenetical mechanisms evolved by "hitchhiking" of secondary adaptations on primary functions of the plant.

7.2.6. Shoot plasticity in response to neighbours; integrating light and hormone signaling

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Plants growing in dense vegetations respond to neighbour plants through shade avoidance responses that include upward leaf movement and increased shoot elongation, thereby enhancing light capture. These responses are traditionally interpreted as phytochrome-mediated responses to reduced red : far-red (R:FR) light ratios of the canopy light.

We show here that several plant hormones, including gibberellins and the gas ethylene, are required to regulate shade avoidance. Ethylene-insensitive tobacco plants are weak competitors for light due to a reduced plasticity to neighbours. This is, however, not entirely related to R:FR signaling as these plants respond well to low R:FR. Interestingly, blue light photon fluence rates are also reduced in canopies and this induces shade avoidance responses in wild-type, but not at all in ethylene-insensitive plants. Furthermore, ethylene could accumulate in canopies to levels that induce shade avoidance responses. We conclude that plant neighbour detection is a complex interplay between different light signals that interact with different hormones, resulting in coordinated plasticity in response to neighbours.

7.2.7. What must a branch say to the plant?

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Branch removal leads to varied developmental modifications, and it follows that an intact branch signals its presence. Both an experimental system of two shoot plants and comparative observations suggest that quantitative information about the local environment of the branch, its size and its developmental rate is carried by the hormone auxin. Information about both the environment and internal developmental relations is thus integrated by being translated to a joint molecular signal.

Auxin and presumably other information coming from a branch elicit responses throughout the plant. These include the oriented differentiation of vascular tissues, along the flux of auxin from its sources in a developing branch. By means of this differentiation the various branches compete, their genetic identity notwithstanding. This competition is a component in the self organization of plant form. Branches also compete for light, which influences auxin formation, again demonstrating that there need not be a separation between internal and environmental information in determining the relations between organs and overall plant form.

7.3.1. Floral syndromes: Empiricism versus typology

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The typological concept of floral syndromes classifies flowers ecologically and naturally according to their pollination agents. Among zoophilous syndromes, generalism and seven specialized (euphilic) flower types are distinguished, characterized by sets of loosely combined attributes. They represent the most superficial and evolutionarily most labile organizational level of the flower, usually recognizable in the living condition only. Determining a syndrome results from an interplay of character analysis and critical field observation. Assignments may be falsified by field data, and these may be misinterpreted by rigorous empiricism. Euphilic syndromes coevolved diffusely with pollinator guilds, resulting in adaptive radiation and world-wide convergences. The present state of classification and the taxonomical distribution of syndromes are reviewed. Although the syndrome concept has a high predictive power and has proved reliable in characterizing taxa, analysing communities, and tracing evolutionary trends, it has not been unanimously accepted. Objections raised, from merely semantic to fundamental, are discussed.

7.3.2. Stamens and mimic stamens as components of floral colour patterns

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Stamens of zoophilous plants are multifunctional floral organs which protect pollen, attach pollen to the pollinators body surface, provide pollen as a reward for flower visitors, and attract potential

pollinators. Pollen eating flower visitors such as syrphid flies and pollen collecting flower visitors such as bees innately respond to stamen and pollen signals prior to individual experience. Flowering plants signalling with conspicuous stamens and pollen to attract pollinators have numerous costs associated with visual exposure of pollen in a way that prevents protection against solar radiation, and against pollen losses caused by wind, rain, and illegitimate flower visitors. Many flowers use mimic stamens (e.g. staminodes, plastic false stamens, yellow floral guides in the size and shape of anthers) and overcome the disadvantages of attracting and rewarding flower visitors with pollen. Several study cases focus on the benefits of signalling with mimic stamens. The aim of this presentation is to illustrate the various cases in which flowering plants may benefit by attracting flower visitors by means of mimic stamens.

7.3.3. Fruit like flowers and pollinators like frugivores: a unique pollination mechanism in *Cullenia exarillata*

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Cullenia exarillata (Bombacaceae) is a dominant tree in south Western Ghats, India. Flowers of *Cullenia* attract the entire contingent of arboreal frugivore community in site, which contribute to its pollination. *Cullenia* showed adaptations to capture their visitors at 3 levels: 1. at individual flower level, they act as fruits and does not have free flowing nectar as it is embedded in the tissues of the sepals, whereas petals are distinctly absent. The visitors remove the sepals and chew it to get the nectar. On many occasions the sepals come out easily as sleeves leaving the reproductive parts intact. 2. The flowers are densely clustered around the lateral branches and exhibit cauliflory. This facilitates elaborate display to attract arboreal mammals. These branches can bear the weight of mammals, which walk on them and help in pollen transfer. 3. Finally, they flower during times of fruit scarcity in the forest that leads to convergence of the entire frugivore community to *Cullenia*. We explore multiple selective factors that could have led to the evolution of this unique pollination system that appears to be between non-flying mammal and bat pollination syndromes.

7.3.4. The evolution of ant pollination systems

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The Hymenoptera are among the most important pollinators worldwide but ants, although abundant in most terrestrial ecosystems, and frequent flower visitors, are rarely involved. I review the evidence for the scarcity of ant pollination and the few cases in which ant pollination has been documented. Two very different systems have evolved: The first, limited to two orchid species with highly specialized interactions involving complex floral traits and insect behaviour, occur only in S. and E. Australia. By contrast, the second kind involves a variety of widely distributed plant families, is relatively unspecialized, with easily accessible floral rewards that ants share with a variety of small winged insects. These systems occur chiefly in harsh habitats where selection for plants with low growth forms favours visits by ants. In the future, more plant species with varying levels of dependence on ants for seed set are likely to be found and the apparently unspecialized systems may yet reveal ant traits that predispose particular species for pollination.

7.3.5. The chiropterophily syndrome: an analysis of the Neotropical species

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Since the revision published by Dobat and Peikert-Holle, in 1985, various other cases of bat pollinated flowers were described. Currently, it is estimated the occurrence of chiropterophily, in the Neotropical Region, in ca. 50 families (among the 65 which have chiropterophilous taxa), 192 genera, and more than 600 species. From these families, 22 are referred to have chiropterophilous taxa only in the Neotropics. In this study we present a revision of published data with chiropterophilous neotropical species, and an analysis of the state of the art of chiropterophily in relation to families/genera and species, with respective floral traits. The syndrome occurs in basal, but also in derived families as Orchidaceae and Fabaceae. Results evidenced case histories with

chiropterophilous species hardly confirming the floral traits referred to the syndrome, with high frequencies of species with white, cream-greenish flowers, of the gullet, brush or bell-funnel types. Recent studies added some features to the syndrome. (Financial support: CNPq).

7.3.6. Chiropterophily in the Cuban Flora

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Cuba, the largest island in the Caribbean, consists of a big variety of landscapes and soils. Together with the geographical position and the influence of tropical climate, these factors contribute to the very diverse Cuban vegetation. With more than 6350 species of angiosperms, half of them are endemic.

Among the Cuban bats there are four species of flower visiting bats. Only one, *Monophyllus redmani*, belongs to the subfamily Glossophaginae, the other three species (*Phyllonycteris poeyi*, *Brachyphylla nana* and *Erophylla sezekorni*) belong to the Phyllonycterinae, a group with a distribution restricted to the Antillean islands.

On the base of an extensive revision of the Cuban flora, we are able to present for the first time a summarizing overview of the potential chiropterophilous plant species of the island. Direct field observations and IR video records, as well as an analysis of the characteristics of floral morphology and phenology of these plants, will be discussed with respect to the syndrome of chiropterophily in general and to potential adaptations to the two different groups of flower visiting bats in the island.

7.3.7. Characteristics associated with wind pollination in the southern heaths (Epacridaceae) - a sub group of the Ericaceae.

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Wind pollination is associated with particular groups including conifers, some monocots and flowering tree species. However sporadically (but frequently) it has evolved in lineages that are predominantly biotically pollinated. Factors which may have forced the change are often associated with extreme environmental conditions that could limit pollinator attention to the flowers of the species. The Epacridaceae has been considered exclusively biotically pollinated, with insects the predominant visitors and vertebrates much less important. However at least three species of *Richea* have a number of characteristics which point towards wind as the main pollen vector. This study outlines some of the characteristics of selected species and evaluates the likelihood that wind pollination has evolved in *R. sprengelioides*, *R. procera*, both in Tasmania and *R. victoriana* in Victoria, Australia. The genus seems to have exaptations, for example the "operculum type" of corolla, which would facilitate development of wind pollination from an animal pollinated ancestor. A number of other floral characteristics of the species conform to the syndrome of wind pollination.

7.4.1. The evolution of anisogamy: an overview

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Anisogamy underlies the important evolutionary processes in sex ratio and sexual selection. Our theory (hereafter PBS) proposed that anisogamy arose through a form of disruptive selection in an ancestral isogamous external-fertilising ancestor, with gamete production under a size/number trade-off. Small gametes (proto-sperm producers) benefit because they can produce many more gametes, parasitically gaining most fusions with the important large gametes (from proto-ovum producers) with a high survival advantage (zygote survival was an increasing function of size). Selective fusions were proposed to have evolved later. Subsequent PBS versions assumed non-random fusions between two mating types. New theories are that: (i) anisogamy is a mechanism for controlling cytoplasmic inheritance of parasitic replicators, and (ii) eggs became larger to increase sperm encounter rates. PBS has been wrongly claimed to require an unusual relationship between zygote size and fitness. A new analysis of PBS shows more clearly the conditions under which the ESS is for isogamy or two sexes: isogamy is most likely in protists, and anisogamy in higher organisms.

7.4.2. Density dependent sexual selection in external fertilizers

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The evolutionary transition from isogamy to anisogamy likely occurred among organisms with external fertilization. While theory offer explanations for this transition, there is little data on sexual selection and whether gamete traits can influence reproductive success in external fertilizers. Using sea urchins and molecular markers, I estimate reproductive success, the variance in reproductive success and the intensity and nature of sexual selection for both males and females under natural conditions in the sea. These measurements are made across a gradient from sperm limited to sperm competitive conditions. The results indicate that (1) gender differences in the intensity of sexual selection is dependent on population density, (2) differences in population density can select for differences in gamete traits that are better adapted to either sperm limited or sperm competitive environments, and (3) there are sex differences in the costs associated with variation in spawning behavior. The nature of sexual selection in external fertilizers is dependent on population density and the targets of selection are gametes and spawning behavior.

7.4.3. Evolutionary origins of anisogamy in marine green algae

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In marine green algae, isogamous or slightly anisogamous species are taxonomically widespread. They produce positively phototactic gametes in both sexes. We developed a new numerical simulation of gamete behavior in C++ to elucidate potential advantages of gamete behavior, with input parameters based on experimental data. Each gamete swimming in a virtual rectangular test tank was tracked and the distances between the centers of nearby male and female were measured at each step to detect collisions. These numerical experiments elucidated the roles of gamete behavior and the mechanisms of the evolution of anisogamy and more derived forms of sexual dimorphism. We found that gametes with positive phototaxis were favored, particularly in shallow water, because they could search for potential mates on the two-dimensional water surface rather than randomly in three dimensions. We also found evidence that sperm limitation is not the dominant selective force in the evolution of isogamous or slightly anisogamous marine green algae; gamete behaviors appropriate to the microhabitat and gamete density appear to be far more important.

7.4.4. Adaptive significance of egg size variation of aquatic animals: referring to mesoscale features of aquatic environments.

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Zygosis rule of megagamete and microgame for initiating ontogenesis of diplophase organisms that is ubiquitous in animals and plants has been a fascinating phenomenon for evolutionary biologists. Anisogamy would be a common resolution for maximizing the chance of making a zygosis and the success of the zygote. Since megagamete (eggs) totally takes on the responsibility of the success of the zygote in anisogamy, we can expect adaptive significance of egg size variation on various environmental conditions. We review egg size variation of various types of fish species inhabiting throughout freshwater habitats and ocean pelagic and demersal habitats by focusing common features of life history in aquatic environment. We further present a theory to understand the adaptive significance of egg size variation of the aquatic animals by focusing the common mesoscale features of aquatic environments, e.g., current, transportation, and environmental gradients.

7.4.5. Why are equally-sized gametes so rare? The instability of isogamy and the cost of anisogamy

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The aim of this study was to determine the circumstances in which isogamy can be maintained in a population that has already evolved mating types. We analysed the evolutionary dynamics of gamete sizes when there are two mating types. The models and conclusions differ depending on: (1) whether size-determining loci are linked to loci-determining mating types or not; (2) whether gamete size affects gamete success or not; and (3) whether viable mutations with large effects on size are possible or not. In all cases, the reproductive success of a zygote depends on the sum of the sizes of the two uniting gametes, and the number of gametes produced is inversely proportional to gamete size. When size is not closely linked to mating type, it is possible for isogamy to be likely stable, particularly when mutations of large effect are deleterious. However, when size is linked to mating type, isogamy can only be stable when there are significant direct effects of size on gamete survival and mating success. The cost of anisogamy is generally less than two-fold.

7.5.1. Reconstruction of the landscape unit relate to the roof-shale level from the Quitéria Outcrop - Rio Bonito formation, Paraná Basin, Rio Grande do Sul State, Brazil - Lower Permian - Gondwana.

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The Quitéria Outcrop is an isolated portion of the Rio Bonito Formation, Paraná Basin - Lower Permian of the Rio Grande do Sul State, Brazil. The present study is an attempt to establish a landscape model from a specific level of this Outcrop. This level is characterized as a roof-shale flora, a singular registry in Gondwana, developed in coastal lagoon depositional system protected by a barrier, composed by abundant autochthonous cormophytic bases of arborescent lycophytes (*Brasilodendron pedroanum*). Also, occur, associated to the autochthonous elements, parautochthonous forms, represented by fronds (*Botrychiopsis valida*, rodheopterideous fronds), conifer shoots with adpressed leaves as well as fertile shoots presenting cones in organic connection (*Cori cladus quiteriensis*), associated to herbaceous lycophyte fertile shoots (*Lycopodites* ? sp.) allowed establishing the presence of canopy forms and understory, representing groundcover and shrub-like vegetation. The study of the floristically components and the Stratigraphic elements are important to reconstruct the landscape unity and to compose an artistically representation presented here.

7.5.2. Permian-Triassic vegetation in Antarctica: Floral turnover in relation to changes in paleolatitude and paleoclimate

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Like the rest of Gondwana, Antarctica experienced a floral turnover at the end of the Permian--from a low diversity *Glossopteris* flora to more diverse assemblages, eventually dominated by corystosperm seed ferns. Floral change occurred throughout the Triassic, reflecting a general climatic warming. The P-T boundary in Antarctica has traditionally been defined by floral change, but evidence of Triassic *Glossopteris* elsewhere in Gondwana calls this definition into question. The best floral records come from the central Transantarctic Mountains (CTM) and Prince Charles Mountains (PCM). In the PCM, mega- and palynofloras show the disappearance of glossopterids and cordaites, followed by the appearance of peltasperms and lycophytes, with corystosperms, conifers and ferns occurring later. In the CTM, the Early Triassic fossil record is poor; palynomorphs indicate the presence of lycophytes, but no corresponding megafossils are known. Floral changes near the boundary will be discussed, along with the increase in floral diversity and abundance from the Early to Late Triassic.

7.5.3. Floristic turnover at the Permian-Triassic boundary in Australia and East Antarctica

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The P-Tr boundary records global devastation of gymnosperm forests in a short transition zone. In Australia and Antarctica this is expressed by collapse in glossopterid abundance and rise in

Lunatisporites pellucidus pollen, locally synchronous with peaks of the fungal/algal palynomorph *Reduviasporonites*. Glossopterid decline is abrupt but other palynofloral changes across the boundary are more gradual. The Early Triassic saw the rise of other gymnosperms, notably peltasperms, voltzialean conifers and corystosperms, many of which were already sparse components of latest Permian floras. This phase is typically associated with a rise in lycophyte spores and/or acritarchs. This floristic transition in Australia-Antarctica is associated with abrupt termination of coal deposition at the P-Tr boundary, sharp decline in carbonaceous shales through the transition zone, and extensive redbeds in the Early Triassic. Complex corystosperm-dominated floras and coal deposition reach a post-extinction peak around Carnian-Norian times. The P-Tr and K-Pg events show similar patterns of plant turnover but the Triassic floristic recovery was more protracted.

7.5.4. Vegetational changes in India during the K/T boundary

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The position of India in the past especially during the Cretaceous-Tertiary was very important as the sub-continent had performed a remarkable northward journey after being separated from other Gondwanaland continents. It would be interesting to know the floral changes taking place on this island continent during the period. During the greater part of the Cretaceous the flora was dominated by the gymnosperms, with few reports of angiospermous fossils of uncertain affinities. The entry of the flowering plants into India might have been late as compared to their appearance on other continents. The definite records of angiosperm fossils started appearing in India from the Lameta beds which are Maastrichtian in age. The successive eruptions of lava flows called the Deccan Traps started in India during the upper Maastrichtian and continued till the Danian with quiescent intervals in between. The sedimentary beds deposited during these intervals are very rich in the plant fossils that reveal a drastic change in the flora during the period. The gymnosperms declined in number whereas the angiosperms which had been poorly represented earlier, suddenly became dominant.

7.5.5. Short-term collapse of vegetation at the Cretaceous-Paleogene boundary in New Zealand - cause for extinction of polar dinosaurs?

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High-resolution palynological investigations of sediments spanning the Cretaceous-Paleogene boundary in New Zealand reveal a sudden turnover in the flora most strikingly expressed by a thin layer of fungal spores coincident with an iridium anomaly of 4ppb. Pioneer recovery vegetation, following the end-Cretaceous catastrophe is represented initially by *Laevigatosporites ovatus* spores succeeded by *Gleicheniidites*, both representatives of ground ferns (Blechnaceae and Gleicheniaceae). Younger assemblages are dominated by spores of tree ferns eg. *Cyatheidites* and *Cibotidites*. Following an interval of fern dominance, gymnosperms and later angiosperms return to the palynological record. Extinction levels of plants do not exceed 15%. The absence of vascular plant spores and pollen and the abundance of fungal spores at the boundary is evidence of wholesale dieback of photosynthetic vegetation due to reduced insolation following the Chicxulub meteorite impact. The period with low light levels is estimated to have lasted less than a year but vegetation dieback had dramatic consequences for the terrestrial food web and on large herbivores in particular.

7.5.6. Floristic changes in the Cretaceous to Cenozoic vegetation of Antarctica

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Recent investigations of patterns of taxonomic turnover and wood abundance reveal little impact of the K-T mass extinction event in Antarctica. This supports previous work on palynofloras that indicate a gradual transition and continued turnover both prior to and after the event. Four stages of forest development are recognized through the Cretaceous and Cenozoic. Aptian to Albian Conifer dominated wood floras are replaced by conifer dominated

but with a presumed minor angiosperm component in the Cenomanian to Turonian. By the Coniacian to Santonian these conifer-dominated forests are replaced by a diverse mixed angiosperm forest. The Campanian to Maastrichtian is characterized by the expansion of Nothofagaceae at the expense of many older taxa. This continues through the Cenozoic till the final extinction in the Neogene. The patterns of vegetation change appear to be strongly linked to climatic fluctuations.

7.5.7. Changes in Cretaceous/Paleogene Angiosperm floras of Southern South America

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It is well known that, while the disintegration of Gondwanaland started during the Mesozoic, the Cenozoic was period of significant geologic activity. Major geological events affected the climate of Antarctica and South America in general, and therefore it affected the distribution and migration of the biota. Here, we re-analyze data gathered from palynology and megafossils in the light of the new evidence and we present examples of how changes in the climate are reflected in the paleofloras. Data collected provides evidence that during the Campanian-Maastrichtian, the angiosperms were dominants in number of taxa but they still were not the dominants in the ecosystems. A remarkable change is observed during the Paleocene-Eocene when the angiosperms are dominant in number of taxa and became the principal component of the ecosystems. Paleofloras of Southern South America had the greatest influence from Antarctica at least until the Eocene, and after that, that influence decreased.

7.6.1. Compositree - where do we go from here?

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Besides the discussion of the state-of-the-art and the way ahead towards the Tree-of-Life for the Compositae within the framework of The International Compositae Alliance (TICA), the presentation will concentrate on a demonstration of potential applications of phylogenetic tree information on groups of this family in the fields of biogeography, evolutionary biology and ecology. Using the Peri-Mediterranean clade of Compositae-Anthemideae as an example, the reconstruction of temporal and spatial aspects of the biogeographical history of this plant group based on a combination of dispersal-vicariance analysis (DIVA) and molecular clock dating of a calibrated, rate-smoothed nrDNA ITS maximum-likelihood tree is demonstrated, along with the employment of phylogenetic information in tests on the relationship between life history trait evolution (e.g. life-form, dispersal structures) and ecological niche differentiation (e.g. growth habit, ecoclimatic conditions).

7.6.2. Molecular phylogeny of the Astereae: Generic relationships among the Daisies, tribe Astereae

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Tribe Astereae with just over 200 genera and 3000+ species are especially well represented in arid temperate, tropical and subtropical habitats with centers of diversity in North and South America, Africa, and Australasia. Unlike the tribe's stable circumscription, subtribal and generic treatments are often paraphyletic and polyphyletic. Many traditionally recognized genera have been shown to be polyphyletic including *Aster*, *Chrysothamnus*, *Ericameria*, *Erigeron*, *Haplopappus*, *Olearia*, *Tetramolopium*, *Vittadinia*, etc. Based on the supertree by V. Funk and colleagues, Astereae is relatively derived and sister to Anthemideae. It appears to have originated in the southern hemisphere, probably in southern Africa or a combination of southern continents, and expanded from there. In conjunction with The International Compositae Alliance, the Astereae Working Group (AGW) was organized to develop a more complete understanding of relationships among Astereae. Initial focus for this effort is to generate a comprehensive, molecular based phylogeny. Information about AGW is given on the website hosted by the New Mexico Museum of Southwestern Biology Herbarium.

7.6.3. Diversification and tribal revision of Helianthoideae

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Molecular studies of subfamily Helianthoideae (Cass.) Lindl. based on several genes of cpDNA reveal 13 lineages we recognize as tribes. The subfamily is primarily characterized by presence of phytomelanin in the cypselae. All lineages of the subfamily have their origin in North America, except for the basalmost lineage, the mostly African tribe Athroismeae. We hypothesize that the evolution of Helianthoideae in America is the result of a long distance dispersal event from Africa. The Helenieae, characterized by cypselae with large crystals and without phytomelanin, is reminiscent of Inuleae and represents the basalmost lineage of the subfamily in the Americas. The next lineage to split is Coreopsidae. The remaining tribes of Helianthoideae are grouped into two main clades. One clade contains Neurolaeneae, followed by Tageteae, and Chaenactideae sister to Bahieae. The other clade contains Polymnieae as sister to two clades, one containing Heliantheae and the other Millerieae sister to Madieae, Perityleae, and Eupatorieae. The present classification scheme is based on the desirability of recognizing monophyletic tribes, including Eupatorieae.

7.6.4. A global molecular phylogeny of the Gnaphalieae (Asteraceae).

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The Gnaphalieae are a tribe of 187 genera (1250 species) of Asteraceae that have their greatest diversity in the southern hemisphere. The objective of this ongoing study is to reconstruct a global phylogeny of the tribe using sequence data from two non-coding chloroplast DNA sequences, the *trnL* intron and *trnL/trnF* intergenic spacer, as well as the *matK* coding region. Included in this investigation are the genera of the Gnaphalieae from the African basal groups, and members of all recognized subtribes. Results indicate that the Relhaniinae, which are restricted to Africa, are not a monophyletic group as presently circumscribed, nor are the South African members of *Helichrysum*, the Cassiniinae and Gnaphaliinae. Also the, primarily Australian, subtribes Angianthinae and Cassiniinae are non-monophyletic as currently circumscribed. In most trees the Relhaniinae s. str. and some of the basal taxa comprise a clade that is sister to the remainder of the tribe Gnaphalieae and furthermore several genera that are currently in the Gnaphalieae, including *Printzia*, *Isoetopsis*, *Denekia*, and *Callilepis*, should be excluded from the tribe.

7.6.5. Origin and diversification of tribe Anthemideae (Asteraceae)

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Tribe Anthemideae (Asteraceae) is composed of 111 genera and approximately 1750 species including many cultivars, such as chamomiles, daisies, and chrysanthemums, and ecologically important species, such as sagebrushes. Molecular phylogenies are largely incongruent with traditional classifications and morphological phylogenies, and instead resolve lineages strongly correlated with biogeographic distribution. The topology includes a grade of many South African genera that are basal to a large clade that is further resolved into two sister subclades. The subclades include 1) eastern Asian genera (including cosmopolitan *Artemisia* and allies) sister to South African genera, and 2) perimediterranean genera (including *Anthemis* and *Achillea*) sister to South African genera. Thus molecular phylogenies support a South African origin for the tribe itself evidenced by the placement of eastern Asian and western Eurasian lineages being deeply nested within and sister to South African genera. Correlations with morphology and karyology will also be discussed.

7.6.6. Reconstructing the evolutionary history of a giant: a preliminary ITS phylogeny of *Senecio* and tribe Senecioneae (Asteraceae)

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Tribe Senecioneae (Asteraceae) includes *Senecio*, one of the largest genera of flowering plants composed of 1,000-3,000 species, which has a cosmopolitan distribution. This genus harbors remarkable diversity in morphology and life-history strategies. Systematic studies in *Senecio* have, however, always been frustrated by its enormous size. To better understand the underlying patterns of diversity and the evolutionary success of *Senecio*, we are inferring its evolutionary history within the broader context of Tribe Senecioneae using DNA sequence data of the ITS region of nrDNA. Preliminary results for over 600 species sequenced thus far support a polyphyletic nature of *Senecio*. Based on these results and morphological characters, we propose a new, monophyletic delimitation of *Senecio* and discuss several interesting global biogeographical patterns in Senecioneae. We are expanding our data set with sequences of several plastid regions and will include representatives of all 150 genera in the tribe and c. 125 sections of *Senecio* in our phylogenetic analyses.

7.6.7. Origin and diversification of tribe Eupatorieae

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Eupatorieae is a species rich group that has long been recognized as a tribe. Thus it was a surprise when initial molecular analyses of the family placed it within Heliantheae s.l. Our estimates of relationships of the tribe, using a data set that has a broader sampling of both gene regions and taxa, shows tribe Perityleae as the sister group. The basalmost diverging lineage within Eupatorieae is formed by *Hofmeisteria*, which shares significant features with Perityleae. Basally diverging lineages of Eupatorieae share the high base chromosome number of $x=18$ or 19 , indicating that the widespread numbers of $x=9$ or $x=10$ are derived. The latter numbers characterize a split within a derived clade of the tribe; the basalmost branches of the $x=10$ clade are formed by taxa such as *Brickelliastrum* and *Carminatia* that have often been included in *Brickellia*, which is $x=9$. Although there is decisive support from molecular data for the dismantling of traditional *Eupatorium*, preliminary results suggest that there will be the need for some adjustments of the subtribal taxonomy.

7.7.1. Multiple phylogeographic approaches to determine the post-glacial colonization history of *Hippophae rhamnoides* L. (Elaeagnaceae) in Europe based on chalcone synthase intron (CHSI) sequence variation

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There are currently strong efforts in plant phylogeographic studies to obtain genealogical data from nuclear DNA gene sequences. Nuclear CHSI sequences were surveyed in populations of *Hippophae rhamnoides* from Europe, where pollen data suggest a post-glacial range expansion of the species into northern Europe from more southern localities. We tested whether sequence-based nuclear genealogies are useful in providing detailed insights into routes of colonization and population demographic changes in this early successional species. We used: mismatch analyses to make inferences about past population growth and the time of expansion; tests for neutral evolution (e.g. Fu's F_s) that can also be useful for assessing deviations from the null hypothesis of population equilibrium; and nested clade analysis. Ideally, one would want to find concordance among the inferences from these different methods, before feeling confident that populations of *Hippophae* have experienced a past history of population growth. However, our results indicate that inferences made from these

different methods are not always congruent, possible reasons of which will be discussed.

7.7.2. Can the genetic structure of plant populations be predicted from species' life history traits?

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Many studies have reported that the way genetic diversity is organised within and among plant populations is related with species' life history traits and geographic distribution. However, most analyses have neglected effects of shared ancestry between taxa although both life history traits and genetic diversity parameters tend to underlie strong phylogenetic inertia. Numerous studies have likewise neglected that many species traits are not mutually independent, which renders the interpretation of detected relationships difficult. Here, we use phylogenetically independent contrast and partial regression analyses to re-evaluate some widely acknowledged relationships between life history traits, geographic distribution and population genetic structure. We include measures of subdivision of diversity based on both maternally and biparentally inherited molecular markers. We conclude that the effect of life history traits on plant genetic structure has been overstated and that the geographical and historical contexts play a considerable role in shaping genetic structures of plant populations.

7.7.3. Biogeography and evolutionary history of *Abies alba* Mill. - a synthesis based on paleobotany and genetics

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Silver fir (*Abies alba* Mill.) is characterized by a complex glacial and postglacial history, which since 30 years is controversially debated for locations of 'effective' refugia, postglacial migration routes, secondary contact and introgression zones. A synthesis based on the combination of data from different fossil signals, macro- and microfossil records, as well as from different genetic marker categories allowed us to clarify some of the debated uncertainties such as the presence of refugia in the Pyrenees, Western Alps and the Northern Apennine, respectively. We put forward a new hypothesis of a glacial refuge in the Western Balkans. The geographic distribution of genetic lineages together with the fossil records shaped large-scale migration routes. Suture and introgression zones were clearly visualised. An attempt is made to unravel evolutionary implications of a species biogeography as a history of migration and genetic diversity.

7.7.4. Phylogeography of European *Asplenium* (Pteridophyta)

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With more than 50 taxa *Asplenium* is the most species rich fern genus in Europe. Half of these rock ferns are diploid and they are ancestral to all European polyploids. Of these 50% are auto- or segmental allopolyploids and 50% are allotetraploids. Most European taxa have distinct distribution patterns and distinct habitat and substrate requirements. We use *Asplenium* as a model system to investigate European phylogeography for cryptogamic plants by exploring the discontinuities in ploidy levels, distribution patterns, breeding systems and genetic diversity in the genus. This paper will compare diploid and polyploid taxa from several polyploid complexes for which we have extensive allozyme and cpDNA data sets (e.g. *A. adulterinum* agg., *A. ceterach* agg., *A. hemionitis*, *A. hispanicum* agg., *A. jahandiezii*, *A. majoricum* agg., *A. petrarchae* agg., *A. scolopendrium* agg. & *A. trichomanes* s.l.) to infer long term glacial refugia and contribute to the debate on phylogeographic patterns in European and Macaronesian plants.

7.7.5. Evolution of zinc tolerance in *Arabidopsis halleri* (Brassicaceae): a phylogeographic approach using cpDNA

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Using hydroponics, we evaluated Zn tolerance for 33 populations of *Arabidopsis halleri*, a pseudometallophyte with metalcolous (M) populations. We showed species-wide tolerance with increased tolerance in M populations. We then used cpDNA variation to determine M population history. We revealed strong genetic structure. However, neither differentiation between edaphic types nor founder effects in M populations have been detected. Isolation by distance was greatly responsible for genetic structure. Moreover, we found genetic differentiation between assemblages north and south of the Alps. Zn tolerance should have appeared early in the species history and may be connected with speciation. M population foundation must have occurred several times and can not be related to population bottlenecks. Therefore, evolution towards increased tolerance should be regarded as convergent evolution in M populations. We suppose at least two glacial refuges for *A. halleri*, on both sides of the Alps. Populations might have survived in northern Europe during the Late Pleistocene and since have diverged from the southern ones.

7.7.6. Intraspecific differentiation, genomic constitution and modes of reproduction of North American genus *Boechea* (Brassicaceae) as deduced from nuclear microsatellite allelic variation

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Genus *Boechea* (= *Arabis* p.p.) constitutes a monophyletic lineage of purely Greenlandic and North American distribution. Hybridization, polyploidisation and apomictic stabilization of new genotypes were shown to be the main factors underlying the high polymorphism encountered in this genus. Application of nuclear microsatellite markers proved especially valuable to analyse patterns of differentiation within and among the three model species *Boechea divaricarpa*, *B. holboellii*, and *B. stricta*. This multilocus marker system revealed introgressions and intraspecific differentiations which have not been seen with nuclear (nrDNA ITS) and chloroplast DNA sequences (*trnL* intron, *trnL/F* IGS). Microsatellite allelic variation was further used, together with data on pollen morphology, to estimate the ploidy levels of herbarium specimens. Additionally, these data were successfully used to correlate modes of reproduction with historic events leading to contemporary geographic distribution patterns.

7.7.7. Immigration of thermophilous key species to Svalbard: Can past patterns be used to predict the future?

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The arctic archipelago of Svalbard was almost completely ice-covered during the last glaciation. The most thermophilous plants occurring there today must therefore have arrived postglacially by long-distance dispersal. As climate models predict an increase of the average temperature in Svalbard, even more thermophilous species may establish, given that they are able to cross the oceans. We study established as well as possible immigrant species to 1) identify the source areas and frequency of previous immigrations, 2) estimate the dispersal potential of species that have not yet arrived in Svalbard, and 3) compare species with different modes of dispersal. A total of 18 widespread plants, including several circumpolar species, have been sampled throughout their distribution range and are subjected to AFLP analysis. We observe regional structuring in all species analysed so far, and it appears that the Svalbard archipelago mainly has been colonized from the east (western Russia).

7.8.1. Recent progress in the systematics of Apocynaceae

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Since the last IBC in St. Louis, progress has been made in almost all parts of the family. In the more plesiomorphic Rauvolfioideae and Apocynoideae, efforts have focused on elucidating tribal circumscriptions. In Apocynoideae, almost all tribes recognized in the most recent classification are not monophyletic, and work is underway by tribe to remedy the situation. In the "Higher Apocynaceae" (Periplocoideae, Secamonoideae, Asclepiadoideae), the situation is somewhat better. Reasonably stable tribal concepts have been established, and for some of the larger tribes, subtribal concepts are emerging. Some of the long-standing disputes about generic limits have been resolved (e.g., *Cynanchum*, *Sarcostemma*); others are about to be solved (*Hoya*, *Dischidia*). The issue of homologies has been clarified in many cases, and hypothesis about their origin have been suggested. Much work remains to be done in all parts of the family, presenting numerous opportunities for young systematists.

7.8.2. Phylogenetic relationships and character evolution in Mesechiteae (Apocynoideae, Apocynaceae)

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Mesechiteae, one of the tribes comprising Apocynoideae, includes ca. 160 species distributed throughout the neotropics. Recent phylogenetic studies resulted in significant changes in its composition and new insights on the relationships of its constituent genera. Thus defined, Mesechiteae comprises four genera dispersed among three subclades and is characterized by four morphological synapomorphies: presence of leaf colleters, style-head with 5 longitudinal ribs, stamens and style-head united by cellular fusion and anther bases non-sagittate. The gynostegium structure provides useful morphological characters within Mesechiteae, supporting the exclusion of *Secundatia*, inclusion of *Forsteronia* and the distinction between *Mandevilla* and the clade formed by *Mesechites* and *Tintinnabularia*. *Mandevilla*, by far the largest genus of Mesechiteae, shows a remarkable variation in morphological traits. Some characters, like the arrangement of leaf and calycine colleters, form of anther base and number of nectaries proved to be phylogenetically reliable, but others, like the shape and size of the corolla tube, are discordant with the current phylogeny.

7.8.3. Progress in systematics of Apocynaceae s. str.

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Of the five subfamilies currently recognized in Apocynaceae, two - Rauvolfioideae and Apocynoideae - comprise the Apocynaceae s. str. Over the past ten years most progress in higher level systematics in Apocynaceae s. str. has been made in the heterogeneous subfamily Rauvolfioideae. During the past couple of years research has focused more on tribal delimitation in Apocynoideae, which are much more homogeneous and with more subtle differences defining tribes and genera than in Rauvolfioideae. These studies have yielded results indicating that the five Apocynoideae tribes recognized in the latest family level classification are not monophyletic and are in need of revision following current criteria and convention. In addition these studies, in agreement with earlier work, show the three subfamilies of the former Asclepiadaceae as nested within the Apocynoideae but failing to form a monophyletic group. Here we present the most important of these new findings in Apocynaceae, particularly in subfamily Apocynoideae.

7.8.4. Relationships in Periplocoideae (Apocynaceae), and the multiple origins of complex morphological structures

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Periplocoideae, a small Old-World subfamily of Apocynaceae s.l., have long been recognized as a group based on the presence of unique scoop-shaped translators (hardened secretions of the stylar head), upon which pollen is shed as tetrads or pollinia. Recent phylogenetic analyses confirmed the monophyly of this group and showed Periplocoideae to be nested within "Apocynoideae", but relationships within the subfamily remained poorly resolved. We

utilized data from morphological characters and sequences obtained from plastid DNA and nrITS in a cladistic analysis of Periplocoideae. The resulting phylogeny allowed us to infer relationships within the subfamily and provided a framework which we subsequently used to explore patterns of morphological evolution in Periplocoideae, focusing on the transition from pollen tetrads to pollinia. Pollinia are found in Orchidaceae and in three subfamilies of Apocynaceae: Periplocoideae, Secamonoideae, and Asclepiadoideae. Our results show that pollinia most likely evolved independently in Periplocoideae, and indicate that there are multiple origins of this complex morphological trait within the subfamily.

7.8.5. *Ceropegia* L. (Apocynaceae-Ceropegieae-Stapeliinae): paraphyletic but still taxonomically sound

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The status and phylogenetic position of most genera within Ceropegieae (48 genera, 770 species) have been verified by analyzing cp and nrDNA datasets. Four morphologically and molecularly sufficiently characterized groups have been newly described as subtribes. The terminal one, the Stapeliinae (containing *Brachystelma*, *Ceropegia*, and all stapeliads), is still not fully resolved with regard to phylogenetic structure and taxonomy. The large genus *Ceropegia*, which is convincingly characterized by its pitfall flowers, is scattered over a grade of clades. One clade is shared by some *Ceropegia* and all *Brachystelma* species, making *Ceropegia* (without *Brachystelma*) paraphyletic. All Madagascan *Ceropegia* taxa investigated and the African *C. robyniana* share a terminal, but mostly unresolved clade with the stapeliads. Thus, again, *Ceropegia* without the stapeliads, is paraphyletic. These results are incongruent with current taxonomy. Because no adequate morphological, anatomical or karyological characters supporting a taxonomical reclassification in accordance with the cladistic analysis were found, maintenance of the current taxonomy is proposed.

7.8.6. Diversification in Neotropical Asclepiadoideae (Apocynaceae)

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Molecular phylogenetics has greatly improved our understanding of relationships in Asclepiadoideae, but studies designed to explain their distribution are still needed. Asclepiadoideae originated in the Old World, probably during the Oligocene. A phylogenetic analysis with available sequences of trnL-F was carried out to estimate the age of the four independent New World clades; taxonomic information and other phylogenetic results were also considered to discuss patterns of diversification. We found a main lineage of New World species that evolved in an isolated South America. This lineage radiated in South America, dispersing northwards and undergoing secondary diversifications in Central-North America. The other three invasions were younger, probably arriving in North America through a northern land bridge in the Miocene. They dispersed to South America before the Panamanian Isthmus connection, soon becoming widespread but not yet rich in species number. Because of their recent origin, diversification of New World Asclepiadoideae seems to be driven by cycles of rapid dispersion followed by regional radiation.

7.8.7. Floral diversity and phylogenetic relationships in subtribe Asclepiadinae (Apocynaceae: Asclepiadoideae)

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A first approximation of evolutionary relationships within Asclepiadinae is inferred from two DNA datasets - ITS, and *trnT-L* & *trnL-F* spacers & *trnL* intron. Both the subtribe as a whole, and the radiation of largely herbaceous genera around *Asclepias* are monophyletic and well supported in all analyses. While resolution within the *Asclepias* complex is limited, some lineages can be identified with confidence. These demonstrate that current generic delimitation is unsatisfactory. *Asclepias* should either be restricted to New World members of the subtribe, or expanded to encompass the entire radiation. In the African species, it is shown that although the corona is taxonomically important for recognising

species, it can be misleading as an indicator of phylogenetic relationships. Vegetative similarities often prove a more reliable guide, but more detailed studies are needed to refine these lineages and to detect morphological characters to aid in their practical recognition.

7.9.1. Taxonomy of monadoid and coccoid green algae: Conflict of classic and modern approaches

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Traditionally monadoid and coccoid green algae were classified in the orders Volvocales resp. Chlorococcales according to the morphological species concept. For example, in the genus *Chlamydomonas* (including *Chloromonas*), one of the largest green algal genera, more than 800 species are described by using only morphological characters of vegetative cells. However, phylogenetic analyses of nuclear-encoded SSU and ITS rDNA sequences of more than 100 strains of both genera have shown that *Chlamydomonas* and *Chloromonas* consists to eight independent monophyletic lineages partly together with coccoid green algae (e.g. *Chlorococcum*, *Tetracystis*) within the CW (basal bodies displaced clockwise) subgroup of the Chlorophyceae. Using polyphasic approaches (e.g. secondary structures of SSU and ITS rDNA sequences, results of crossing experiments, sporangium autolysin data and studies of life cycles), a new generic and species concept within the CW-subgroup (traditionally designated as "Volvocales" and "Chlorococcales" s.str.) can be designed demonstrated here by "*Chlamydomonas* and its relatives".

7.9.2. Toward natural systems of species/genera of microalgae based on the comparative light and electron microscopy and robust phylogenetic results

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Species/genera of the microalgae were traditionally classified based on the light microscopical attributes that the phycologists believed important. However, such characters may be unstable and it is very difficult to identify species in some microalgal genera. Furthermore, recent molecular phylogenetic analyses demonstrated that morphologically delineated taxa may be nonmonophyletic. Therefore, comparative light and electron microscopy of cultured materials are needed to obtain stable taxonomic characters, and such characters should be examined based on the robust phylogenetic results in order to construct natural taxonomic systems of microalgae. Such studies at species level have been carried out in the green algal genera *Chlorogonium*, *Vitreochlamys*, *Asterococcus* etc. However, natural classification of genera needs robust phylogenetic results that are not generally resolved using only single-gene information. We recently carried out multigene phylogeny (6021bp) of the advanced members of the colonial Volvocales (*Eudorina*, *Pleodorina* and *Volvox*) and divided *Pleodorina* into two genera based on the new morphological diagnosis.

7.9.3. Phenotype versus genotype in Chlorellaceae

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Classical approaches using morphological characters for the circumscription of taxa do not adequately reflect the phylogenetic relationships. According to traditional systematics, *Chlorella* (spherical, solitary, without spines and mucilage) and *Micractinium* (spherical, mostly in coenobia, with spines and mucilage) belong into different families of the Chlorophyta: Chlorellaceae and Micractiniaceae. However, investigations on 18S rRNA phylogeny revealed a very close relationship of the two genera. Using culture medium of *Brachionus* phenotypic plasticity in *Micractinium* was studied. Unspined *Micractinium* cells can develop spines during incubation with *Brachionus* culture medium. These results suggest that morphological characteristics like spines formation represent phenotypical adaptations to the conditions in the ecosystem. A genus and species concept in Chlorellaceae remains provisional, because there is still a lack of combination of classical and molecular approaches to identify clear phylogenetic lineages.

7.9.4. Phenotypic plasticity in algae: useful rather than problematical

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Algae have successfully colonised aquatic, terrestrial and subaerial habitats world-wide. As a consequence of their diverse ecology, algae have evolved a variety of mechanisms for adapting to changing environments. One such mechanism is phenotypic plasticity. This occurs when a genotype produces morphological alternatives, through differential gene expression, when exposed to different environmental conditions. Despite overwhelming evidence of phenotypic plasticity in algae, the implications for taxonomy, ecology and evolution have not been adequately recognised. Examples will be given to show how phenotypically plastic algae can be used as “model organisms” to elucidate the systematics, ecological and phylogenetic relationships of +algae, by combining culture studies with high resolution microscopical techniques (Scanning Electron Microscopy, Transmission Electron Microscopy, Confocal Laser Scanning Microscopy), biochemical, physiological, and/or molecular analyses.

7.9.5. Coccoid trebouxiophytes dominate green algal biofilms on air-dry artificial hard substrates

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The green algal class Trebouxiophyceae comprises mainly coccoid algae from subaerial habitats. Its monophyly is still challenging and division into rDNA clades is incongruent with morphology-based concepts of families/orders. Biofilms on air-dry artificial substrates (e.g. roof tiles) are an excellent habitat to further investigate the diversity of the class and provide an expanded taxon sampling with which its phylogeny can be better addressed. DGGE, rDNA cloning and sequencing revealed a high diversity of trebouxiophytes on such substrates and was higher than with morphospecies alone. For many of the discovered phylotypes there were no close sequences available or if available, identification was unequivocal at genus/species levels (reference sequence were from unidentified species or polyphyletic genera). Within the same morphotype distinct “cryptic” species were found, but the combination of 18S/ITS rDNA sequencing with AFLP genotyping allows the molecular definition of species. However, also re-investigation of morphological characters is required and the molecular phylogenies point to key taxa which may exhibit important characters.

7.9.6. Pioneer aerophytic microorganisms on outer building surfaces - a comprehensive approach

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Biological growth on façades and other external building components became more evident in recent years and is increasingly recognized as a topic of scientific and industrial concern. There is still significant uncertainty regarding the composition, the taxonomic structure of different types of microbiological growth and their ecophysiological and environmental prerequisites. In addition to biological research, chemical/physical processes are investigated and documented as a part of interdisciplinary collaboration at the Fraunhofer Institute for Building Physics (IBP). Influences of global change, modifications in construction as well as alterations in the composition of air pollution are discussed. Based on the taxonomic differentiation of observed biological communities the conditions for the development of pioneer growth can be assessed. More than 120 different taxa have been documented and investigated as essential colonisers of external building components so far.

7.9.7. Are there really conflicts between classical and modern taxonomy in coccoid green algae?

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Since centuries algal species were defined on morphospecies (“classical”) concepts. Light microscope studies of field samples still do need such concepts, as far as possible with help of

cultures. The development of molecular biological research lead to a phylogenetic (“modern”) species concept reflecting evolution patterns, affinities and discrepancies in morphologically homogenous taxa (e.g. *Chlorella*). This raised problems for a practicable species taxonomy applied in algal floras. Variability in form and function of many algal taxa is still poorly known (e.g. *Oocystis*) but defined structural and morphological data are required for a modern taxonomy in the same extent as a molecular defined status. Some pathways for a modest “polyphasic taxonomy” connecting the classical and the modern concepts as applied in the “Süßwasserflora von Mitteleuropa” are presented.

7.10.1. Mechanisms of rRNA gene silencing

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Allopolyploidy is a significant evolutionary process, resulting in new species bearing sets of chromosomes from two or more progenitors. One consequence of genomic merger in *Arabidopsis suecica*, the allotetraploid hybrid of *A. thaliana* and *A. arenosa*, is dominance of the *A. arenosa* rRNA genes and silencing of *A. thaliana* rRNA genes. This phenomenon, known as nucleolar dominance, involves an epigenetic switch mechanism in which concerted changes in promoter cytosine methylation and histone modifications define the on and off states of the rRNA genes. Using transgene-induced RNAi to knock down whole families of chromatin modifying activities, we have identified a number of modifier genes required for uniparental rRNA gene silencing, including both DNA and histone modifying activities. Our progress in understanding how DNA methylation and histone modifications are interwoven to regulate rRNA gene transcription and NOR condensation/de-condensation will be presented.

7.10.2. Nucleolar proteins and the structural organization of preribosomal RNA synthesis and processing in the nucleolus

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The nucleolar protein NopA100 was identified as a major component of the soluble fraction of nuclear proteins from onion root meristematic cells. This fraction contains RNPs active in the nuclear RNA metabolism. NopA100 is the most phosphorylated protein of this extract, but it is also present in the nuclear matrix. When detected *in situ*, it showed a preferential nucleolar location in the fibrillar components, where the early pre-rRNA processing takes place. Using microscopy and Western blotting, a correlation of the levels of NopA100 and its phosphorylation with the nucleolar activity, the cell proliferation status, and the cell cycle progress, was observed. NopA100 undergoes a physiological process of proteolytic maturation, associated with the increase in pre-rRNA transcription and processing and, consequently, with the activity of the protein. This activity was the upmost in the G2 phase of the cell cycle, when ribosomal genes reach their highest transcriptional rate. NopA100 is a plant homologue of mammalian nucleolin and its features reflect the close relationship of the structure and function of the nucleolus to cell proliferation.

7.10.3. Organisation and evolution of *Solanum* rDNA and nucleolar dominance in allopolyploids

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In the genus *Solanum*, amplification/deletion of subrepeats in the intergenic spacer (IGS) was the main event in the molecular evolution of the 35S ribosomal RNA genes (rDNA). Three species, *S. lycopersicum* (tomato), *S. tuberosum* (cultivated potato), and *S. bulbocastanum* (wild Mexican potato) demonstrate prominent differences in the number/organization of repeated elements upstream and downstream of the transcription initiation site (TIS). Uniparental activity of the 35S rDNA is observed in interspecific hybrids. To test whether the preferential transcription of the parental rDNA could be influenced by the repeated elements, expression of parental rDNA was evaluated in somatic hybrids between the three *Solanum* species and in back-crossed plants. An expression hierarchy was found: In leaves of the respective allopolyploids, rDNA of *S. lycopersicum* dominates over rDNA of *S.*

tuberosum, whereas rDNA of *S. tuberosum* dominates over that of *S. bulbocastanum*. Hence, in the allopolyploids of *Solanum* evolutionary novel rDNA carrying more repeated elements downstream of TIS is transcriptionally dominant over the rDNA with ancestral organization.

7.10.4. Evolution and expression of ribosomal RNA genes in two allopolyploids of recent and recurrent origin

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We have investigated copy number and expression of parental ribosomal RNA genes in multiple populations of *Tragopogon mirus* and *T. miscellus*, two allotetraploids that formed recurrently within the last 80 years following the introduction of three diploids (*T. dubius*, *T. pratensis*, and *T. porrifolius*) from Europe to North America. In most present-day populations of both tetraploids, the rDNA of *T. dubius* origin is reduced and may occupy as little as 5% of total rDNA in some individuals. However, in two populations of *T. mirus* the general trend was reversed indicating bidirectional concerted evolution within a single species. In plants having a low rDNA contribution from *T. dubius*, the rDNA of *T. dubius* origin was nonetheless expressed and *T. dubius* transcripts often outnumbered transcripts made from the partner genome. We detected cases of no, partial or complete nucleolar dominance within a single species; in one population of *T. mirus* the rDNA expression patterns varied even among the progeny of a single plant suggesting that rRNA gene silencing may have limited stability in these recent allopolyploids.

7.10.5. Suppressed concerted evolution and main traits of reticulation in agamosperous polyploid dandelions (*Taraxacum*, Compositae)

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In mainly agamosperous *Taraxacum* reticulation combined with polyploidization represents one of the major evolutionary step. We analysed the association between dandelions ITS1& ITS2 sequence polymorphism, agamospermy, polyploidy, hybridization, and the phylogenetic information. To characterize intragenomic variation and to readdress the evolutionary impact of hybridisation in *Taraxacum* we sequenced ten clones from a single individual of *Taraxacum* sections *Leucantha*, *Suavia* and *Stenoloba*. Main results of our study are: (1) Reticulation is an important process in the evolution of *Taraxacum* and ITS data have proven useful for reticulate phylogeny of this intricate complex. (2) Sequenced alleles display a complex pattern of different ITS repeat types and putative recombinants among repeat types that persist due to lack of concerted evolution across loci or at least its reduced rates. (3) The position of cloned spacer sequences in the tree revealed reticulated network among C Asian *Taraxacum* and suggest hybridisation from lineages of sect. *Leucantha* to sections *Suavia* and *Stenoloba*.

7.10.6. rDNA loci as chromosomal markers in studies of plant development and phylogeny

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The rRNA gen loci, present and express in each cell as housekeeping genes, offer several advantages for cytogenetic and molecular studies. The number and distribution of 5S and 18S-5.8S-26S rDNA loci provides useful markers of chromosomes to study genome evolution and chromosomal variation. Comparative analysis of the distribution of rDNA sites in chromosomes of allopolyploids and their diploid ancestors is a valuable indicator of evolutionary dynamic. The number and distribution of rRNA loci in chromosomes of individual plants indicates intraspecific variation or their structural and numerical rearrangements. Similarly, the level of polyploidization and/or DNA amplification in cells during ontogenesis or plant culture *in vitro* can be evaluated in interphase nuclei through the number of rDNA sites. The variation in the number of rDNA loci in diploid and polyploid species of

Brassicaceae family, the genus *Chenopodium* and in liverwort species, detected through fluorescence *in situ* hybridization, will be presented and the processes leading to reorganization of genomes will be discussed.

7.10.7. 5S rDNA transcription regulation in *Arabidopsis thaliana*

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We are interested in the epigenetic mechanisms involved in the regulation of the polymerase III-transcribed 5S rDNA in *Arabidopsis thaliana*. 1000 copies of 5S rDNA units are organized in tandem arrays present in heterochromatic pericentromeric regions of chromosomes 3, 4 and 5. In the transcribed 5S rDNA loci, the major 5S rDNA genes are expressed whereas the minor ones (containing one or two mutations) are repressed. In leaf nuclei of mature plants, the transcribed fraction makes loops that emanate from chromocenters. These loops which are enlarged in mature ddm1 nuclei are enriched for histone H3 acetylated at lysine 9 and methylated at lysine 4 compared to the heterochromatic chromocenters. Results obtained with the remodeling chromatin mutant ddm1 have shown the involvement of the chromatin structure in the repression of the minor 5S rDNA genes.

Up to four days after germination, heterochromatin is not fully developed, the 5S rDNA resides in pre-chromocenters, does not form conspicuous loops and minor 5S RNA are present. All together, our results indicate that expression and chromatin organization of 5S rDNA genes change during chromatin establishment.

7.11.1. Are methodological constraints limiting our knowledge on dispersal?

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Dispersal types of diaspores are commonly categorized by the vectors transporting the diaspores. Most often these dispersal types are derived from diaspore morphology. Diaspore morphology, however, affects only one aspect of dispersibility, dispersal potential which may be defined as the probability of diaspores to be dispersed over long distances. However, recent studies are questioning the often presumed overriding effect of commonly used morphological traits on dispersal potentials. Diaspore production, the availability of dispersal vectors in both, quality and quantity, as well as differences in dispersal kernels may override the effect of diaspore morphology.

Several dispersal type spectra of large species sets, calculated using different methods for assessing dispersal types, calculated using different methods, will be compared. The methods comprise a morphological approach, field and lab-experiments, a rule based approach and a database approach. Besides detected methodological differences, the results suggest that on the landscape level dispersal type spectra are predominantly regulated by the availability of dispersal vectors.

7.11.2. Directed dispersal selects for mechanisms that ensure germination under nurse plant shade

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In the Kalahari an assemblage of fleshy fruited perennials and annuals grows mainly under acacias. Seed rain is concentrated under trees due to the behaviour of their dispersal vectors, birds and ungulates. We hypothesize that canopy species have developed mechanisms to germinate only under well shaded conditions because under canopies the quality of shade varies spatially. We tested this for diurnal temperature amplitude (DTA), the duration of moist conditions and soil moisture perse. Germination of all fleshy fruited canopy species was inhibited at high DTA compared with constant temperature or low DTA. Germination of annual canopy species was reduced by high DTA in only two species. In the other species DTA had a positive or no effect. We found a significant correlation between germination lag and canopy association. However, this was not significant when data were corrected for the effect of ingestion. No relation between canopy association and germination response to a moisture gradient was found. We predict that the use of DTA to detect optimal canopy conditions for germination is widespread among bird dispersed species depending on nurse plants.

7.11.3. Can evolutionary age, colonization and persistence ability explain to which extent species fill their potential range?

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We test whether the extent to which a species fills its potential range depends on evolutionary age, colonization and persistence ability. For 37 species of Proteaceae, we estimate potential range sizes using bioclimatic models, evolutionary age using molecular phylogenies, persistence ability using field based estimates of longevity, and colonization ability using a process based seed dispersal model, the spatial arrangement of potential habitat, and data on local abundance. We find, in most of 180 alternative phylogenies, a phylogenetically independent increase of range filling with colonization ability. Moreover, range filling increases more rapidly with colonization ability for persistent species. Evolutionary age and contemporary abundance do not explain a significant proportion of the residual variation in range filling. We conclude that, although our analysis provides evidence that range dynamics of the study species can be described by metapopulation models, the dynamics appear too complex to be well described by the simple Levins metapopulation model.

7.11.4. Dispersal limitation in bird-dispersed species: bridging the seed rain and the seedling recruitment stages. A Mediterranean highland study at a community level

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Dispersal limitation exists when the number of recruiting individuals at a given site is constrained by the availability of delivered seeds. In bird-dispersed species, an unequal delivery of seeds to different microhabitats might saturate some of them leaving others underexploited; in this context, dispersal limitation could operate, depending on microhabitat quality. We studied the relationship between seedfall and seedling emergence for a fleshy-fruited plant community at a landscape scale, in the same years (2-3 years) and sites (considering 6 distinct, patchily-distributed microhabitats), in a Mediterranean highland ecosystem of SE Spain. We set seed traps to sample seed rain, estimated seedling recruitment in adjacent plot, and analyzed their spatial concordance at an overall basis as well as the differences for this relationship among the species and microhabitats considered. The importance of the transitional stages between the seed rain and the seedling emergence stages is discussed.

7.11.5. Spatio-temporal dynamics of seedfall and seedling emergence in animal-dispersed species: bridging dispersal and establishment

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Seed dispersal creates the initial template for the regeneration of plant populations. Particularly in perennial plants, it is also the most variable and least predictable stage of the life cycle. Given the typically very small probability that a given dispersal event will result in establishment of an adult plant, long-term studies are therefore required to reveal which microsites in the landscape receive consistently more seeds than others, and if this consistency results ultimately in the existence of recruitment 'hotspots'. We explore patterns of year-to-year consistency in the spatial distributions of seedfall and seedling emergence of three animal-dispersed woody species growing in contrasting habitats. Among-year variation of seedfall across microsites is largely determined by two factors, the crop size of fruiting plants ('source component') and the pattern of dissemination resulting from disperser activity ('dispersal component'). We dissect these two factors and quantify their respective influence on the spatial and temporal consistency of seedfall and seedling recruitment patterns.

7.11.6. Flood dispersal and establishment limit distribution of *Juncus atratus* on different scales

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Juncus atratus is a perennial from regularly-flooded river corridors. Populations are patchily distributed on both local and regional scales. Suitable habitats also show a patchy distribution. Only a fraction of them are colonized. To which extent do germination, establishment and dispersal limit colonization on population, local and regional scales? Germination rate of seeds is sufficient under various conditions, indicating that germination is never limiting, given sufficient water supply. Germination is best in water. Seeds have high floating ability and can thus be dispersed over long distances. Many may germinate when floating. These seedlings, however, can establish only shortly after germination, which limits long-distance dispersal by water. Establishment is also poor for seedlings emerging in turf, indicating site-specific limitation. We conclude that establishment limits both colonization on the smaller scales and long-distance dispersal by water.

7.12.1. Vascular epiphytes as indicators of changing environmental conditions

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Certain Cloudforest formations are seriously threatened by two human impacts which influence the overall hydrological cycle, namely climate change and deforestation. Epiphytes represent an important community which are poised at the interface of forest canopy and the atmosphere, and so provide a remarkable model system to investigate the impact of a changing climate on water and nutrient availability and use. Stable isotopes act as markers of photosynthetic limitation (C), nutrient sources (N) as well as water source and transpiration (18O, Deuterium). Most recently, by using these isotopes in combination, we have the potential to provide additional on the physiological dynamics of these populations. For instance, isotopes can be used to partition water sources (whether meteoric or occult precipitation via 18O) and carbon gain (via 13C) and so the historical record of plant material in herbarium specimens could provide a proxy for recent periods of rapidly changing climates. Additionally, we need to review the status of epiphyte communities, whether by examining the annual changes in standing biomass components or by following recovery of populations after disturbance and deforestation. The aim of this introductory talk is to review the latest developments and provide a platform for collaborative proposals developing more integrated research programmes for the future within this important area.

7.12.2. Stable isotopes as integrators of exposure and habitat preference in epiphytic bromeliads

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Epiphytic bromeliads are subject to intermittent precipitation inputs, such as rainfall and fog, which can be exploited over varying timescales, depending on seasonality, growth form (use of tank and/or trichome), and exposure within the forest canopy. By comparing leaf-water and organic material 18O signals, as well as 13C composition, along an altitudinal and latitudinal gradient between Panama and Mexico, we relate the habitat preference of C3 and CAM bromeliads to exposure, and seasonality of precipitation inputs. We examine the 18O isotopic relationship between precipitation inputs, leaf water and variation along the monocot leaves, and the analysis of water vapour to distinguish isotopic enrichment due to evaporation and transpiration. We will show how epiphytes act as sensitive markers of changing environmental conditions and could be used throughout the neotropics to characterise environmental gradients and changing climatic conditions.

7.12.3. Gender in the mistletoe *Loranthus europaeus* - females work harder

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The mistletoe *Loranthus europaeus* is trioecious with small flowers, which is a rare exception among the Loranthaceae, which

usually have brightly coloured, bird pollinated hermaphroditic flowers on the same plant. In *L. europaeus* the population of hermaphroditic plants is functionally male, as almost all flowers are shed once the pollen is spent and very few fruits develop. Male and female plants differ in their morphology and physiology. Male plants are more ortho geotrophic and stout and less pendulous than female plants. Water potential is lower in female plants when compared to male plants under the same conditions. Autumnal leaf abscission is significantly later in females. This indicates that female plants have to work harder to support fruit development and maturation. Foliar element analysis as well as stable isotope analysis is currently performed and the results will be presented at the congress.

7.12.4. Population dynamics of epiphytes related to host branch characteristics

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The demography of epiphytes is complicated by the fact that they live in a three-dimensional space defined by their host trees with strong gradients of resources and substrate stability. We studied populations of several orchid and bromeliad species in a Mexican humid montane forest over several years and modelled their population dynamics using matrix models. Proportional odds ratio models showed an effect of exposure or branch type on the probabilities of survival and of reaching reproductive size. Mortality decreased with increasing branch exposure in the orchids *Jacquinella leucomelana* and *J. teretifolia* and the bromeliad *Catopsis sessiliflora*, which are common also in more open or disturbed vegetation, but increased in the orchid *Lycaste aromatica*, which is restricted to closed forests. In most species, survival increases with the bryophyte cover on the branch, and population growth rates depend mainly on survival rates of reproducing plants. Matrix projection suggests that under the current environmental conditions several populations - especially that of *Lycaste aromatica* - are declining and will be nearly extinct within one hundred years.

7.12.5. How much do we learn from ecophysiological studies with vascular epiphytes about the compartment of populations in a changing world?

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A principal question of ecophysiology is how physiological attributes contribute to survival, growth and reproduction under the particular habitat conditions of a species. The underlying assumption, however, i.e. that physiological limitations translate into a similar role in populations, has rarely been tested. I present physiological and long-term demographic data for the epiphytic bromeliad *Werauhia sanguinolenta* that show that the scaling from ecophysiology to demography is not straightforward. E. g., although water stress has been identified as the principal abiotic limitation for epiphytes, population growth did not vary with annual precipitation over a 7-year period. On the other hand, the most plastic response to resource availability was found in smaller plants, both in physiological experiments and demographic observations. Discrepancies arise because host tree dynamics (from small twig to entire tree) may influence epiphyte population dynamics more than abiotic constraints. Hence, global change may affect epiphytes not only due to a change in climate, but at least as much due to the proposed increase in the dynamics of tropical forests.

7.12.6. Evolution of epiphytism and crassulacean acid metabolism in Bromeliaceae in relation to changing environments and palaeoclimates

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The family Bromeliaceae represents one of the most distinctive and ecologically diverse groups of vascular epiphytes. Approximately half of the 3000 species are epiphytic and show one or more adaptations that have enabled colonization of xeric microhabitats, such as water-impounding phytotelmata, absorptive epidermal trichomes, and possession of the crassulacean acid

metabolism (CAM) mode of photosynthesis. Phylogenetic reconstruction indicates that both the epiphytic habit and CAM photosynthesis have arisen multiple times in the family's history. In subfamily Tillandsioideae, the epiphytic habit preceded the evolution of CAM photosynthesis, whereas CAM photosynthesis and the terrestrial habit are plesiomorphic in subfamily Bromelioideae, with the epiphytic habit appearing subsequently. In the almost complete absence of a fossil record for the family, analysis of molecular-phylogenetic trees based on nucleotide sequence data from appropriate loci can be used to delimit the timing of the origins these key functional traits, and to consider their possible relation to changing environmental conditions during the Tertiary.

7.12.7. Neotropical epiphyte diversity - present patterns, past causes, and lessons for the future

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We present an overview on Neotropical epiphyte diversity at different spatial scales, based on a biogeographic analysis of plot inventories and local and national checklists of vascular epiphytes. The main focus of our study was the dependence of epiphyte diversity and endemism on elevation and climatic factors. The influence of elevation and rainfall on epiphyte diversity is strongly scale dependent. On local and national scale, both diversity and endemism of vascular epiphytes reach a maximum at mid elevations in Andean cloud forests. Amount and seasonality of rainfall are the major determinants of epiphyte diversity for local inventories of lowland sites. Plot diversity is only weakly correlated with rainfall and not with elevation, with the most species-rich plot located in Western Amazonia. We discuss reasons for different diversity patterns at different spatial scales and the role of the Andean orogenesis as well as past and present rainfall patterns in the generation and maintenance of epiphyte diversity in the Neotropics. Potential implications for priority settings of protected areas in the light of future climate change are given.

7.13.1. Functional chemistry of leaf surfaces

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Leaf surfaces are the interfaces between the physiologically most active tissues of higher plants and the abiotic and biotic environment. Primary plant surfaces are covered by a cuticle which acts as a signal in epidermal cell differentiation, as a transport-limiting skin and as a substrate for biotic interactions. The symposium will assess the state of knowledge on how functions are related to the chemical composition of plant cuticles. Major contributions will cover the role of cuticles in epidermal development and differentiation, the chemical basis for the barrier function of plant cuticles, and the physical properties and ecological relevance of epicuticular waxes. Further topics will include the role of cuticular waxes and cutin constituents in plant/microbe and plant/insect interactions. The objective of the symposium is to advance the integrative view of plant cuticle chemistry and function, and to lay the foundations for a modern biology of plant surfaces.

7.13.2. Wax biosynthesis and cuticular permeability

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Cuticular waxes play a pivotal role in limiting transpirational water loss across all primary above-ground plant surfaces. The relationship between the chemical composition of the cuticular waxes and its function as a transpiration barrier as well as their biosynthesis, however, is still unclear. It is the aim of our group to correlate all these three parameters using one single plant system. Intact tomato fruits (*Lycopersicon esculentum*), due to their astomatous surface, are an ideal model system to integrate this biosynthesis-composition-function relationship. Additionally, tomato is an important crop with sufficient genetic and genomic tools available. Using mutants with genetically manipulated cuticular wax compositions are analysed for their transpiration barrier properties. One mutant with significant changes in the composition of very-long-chain aliphatic compounds due to a gene knock-out in a fatty acid elongase -ketoacyl-CoA synthase showed a dramatic increase in cuticular transpiration. Recent progress in this project will be presented.

7.13.3. *Arabidopsis* requires signal pathways for the formation of epidermal cuticle

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Leaf epidermis of plants plays an important role to restrict permeability of water-soluble molecules across the surface of organs and inhibit unfavorable adhesion between organs. To exert the epidermal function, it is necessary to express genes involved in biosynthesis of components for cuticle, establish the polar transport of the components and to deposit the cuticle on the outer surface. Using molecular-genetic approaches involving a new method to visualize defects in cuticle [1], we have identified several genes that control proper formation of cuticle on epidermal and protodermal surfaces of *Arabidopsis thaliana*. They include the *ABNORMAL LEAF SHAPE1* (*ALE1*) gene encoding subtilase (putative serine protease) [2], the *Arabidopsis* homologue of the maize *Crinkly4* gene (*ACR4*) encoding a receptor-like protein kinase [3], and the *ABNORMAL LEAF SHAPE2* (*ALE2*) gene encoding a receptor-like protein kinase as well as genes for presumptive metabolic enzymes for condensation of fatty acids [1]. Mutations in these genes cause various morphological defects in all the plant organs including abnormal shapes of embryos and fusion between leaf-like organs. Shapes of protodermal and epidermal cells of *ale1*, *acr4*, and *ale2* mutants are also rudimentary. Electron microscopic analyses show partial loss of a cuticle layer on the surfaces of these cells. Although loss-of-function mutations in any one of *ALE1*, *ACR4*, and *ALE2* genes exhibit relatively weak epidermal defects, mutations in *ALE1* and *ACR4* or those in *ALE1* and *ALE2* result in markedly severe defects in morphology of epidermal cells of seedlings and in cuticle on the epidermal surface. The above results suggest that these genes play critical roles in the formation of proper cuticle. We propose that at least two independent pathways, each including *ALE1* and *ALE2/ACR4*, might control the formation of epidermal cuticle in *Arabidopsis*.

7.13.4. Digestion of the cuticular barrier leads to pathogen defence

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Components of the cuticle such as hydroxylated fatty acids were shown to be potential elicitors for defence reactions (Schweizer et al., Plant J., 10, 331-341, 1996). This was further tested in our group by overexpressing a cell wall-targeted fungal cutinase of the pathogen *Fusarium solani* in *Arabidopsis thaliana* under a constitutive or an inducible promoter. Transformed plants have a reduced cuticle and exhibit an increase in resistance to the necrotrophic fungal pathogen *Botrytis cinerea*. Experiments were undertaken to understand the basis of this counterintuitive result. Compared to controls, cutinase-expressing plants showed changes in gene expression (determined by DNA microarray analyses) indicating sensing of the surface defects. Amongst others, the genes of polygalacturonase-inhibiting proteins, a potential barrier to *Botrytis* penetration, were induced. The direct toxicity of the fungal cutinase, its products, or other components leaking out at the surface of the transformed plants was also tested. Among these, only yet uncharacterized components showed a fungistatic effect against *Botrytis*. These components will be analyzed further.

7.13.5. Leaf epicuticular wax signals for pathogen specificity

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The leaf epicuticular wax represents a protective layer against pathogens. Conversely, it may provide the first signals involved in host plant recognition to a specialized pathogen. Chemically, it mainly consists of cutin and wax esters matrix, also including terpenoids and phenolics in its inner structure. This composition also affects the micromorphology of the layer. Several studies pointed out that constituents of the cuticle influence germination and appressorium formation of pathogens. Our experiments with *Pyricularia grisea*, the causative agents of rice blast disease, and *Erwinia amylovora*, the fire blight on apples,

suggest that detection of stimulatory and inhibitory effects of leaf epicuticular waxes are influenced by the choice of extraction solvent. Different extraction capacities of various solvents decisively affected the extract activity. The exemplification of such signals might contribute to the elucidation of host recognition mechanisms of pathogens.

7.13.6. Plant surfaces from the insects' view

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When phytophagous insects alight a plant, they first encounter the species-specific chemical and morphological structure of the cuticular surface which can be used as cue for discrimination of susceptible from non-acceptable hosts. To test the influence of the plant cuticle on feeding and oviposition, different bioassays were developed in which side-specific characteristics of leaf chemistry and morphology were considered. Due to the degree of specialisation, insects may respond differently to wax cues of the same plant species. Presence of epicuticular waxes was crucial for oviposition acceptance in a monophagous leaf beetle, while some oligophagous species did not discriminate between leaves with and without intact wax layer. In contrast, a generalist leaf beetle was shown to prefer de-waxed leaves of this plant which was a poor quality host for its development. The wax layer can serve as recognition cue, but might also cover feeding stimulants of the inner plant tissue.

7.14.1. Ecological linkages between soil and above-ground communities

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Soil biological communities are intimately linked to the plant community. The increasing recognition of the influence of these components on each other has led to there being much interest amongst ecologists in the role played by aboveground-belowground feedbacks in controlling ecosystem processes and properties. This talk will use selected examples from recent studies to explore the nature and ecological significance of feedbacks between plants and soil biological communities in relation to soil nitrogen and carbon cycling. The talk will also examine recent studies that address the question of whether variations in the diversity of soil biological communities are of significance for these feedbacks between plants and soils. The overall aim of the talk is to illustrate the importance of soil biota and their interactions with plants as major structuring forces in plant communities.

7.14.2. Earthworms as ecosystem engineers in below-aboveground interactions

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Earthworms play a mayor role in nutrient cycling and have pronounced impacts on plant growth. We studied the effects of earthworms in combination with soil heterogeneity and other soil organisms (mycorrhiza, plant feeding nematodes) on growth, competition and defensive chemistry in plants, and the consequences on aboveground herbivore performance. The experimental plants were a grass (*Lolium perenne*), a non-legume forb (*Plantago lanceolata*) and a legume (*Trifolium repens*). Due to different root morphologies and N acquisition strategies the plants responded differently to earthworms. In *P. lanceolata* we found changed levels of defense related compounds (iridoid glycosides, phytosterols) in the presence of earthworms. Since effects of earthworms on plants are mediated by nutrient availability and microorganisms, the effects vary under different soil conditions. By changing the food quality of plants, earthworms can thus affect aboveground herbivore performance.

7.14.3. Interactions between insects and arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi are ubiquitous in nature and associate with the roots of about 75% of all vascular plants. The association is considered to be mutualistic, because the mycorrhiza donates mineral nutrients to the plant in return for a supply of organic carbon. Both of these features mean that a mycorrhizal plant may represent a very different food source for an insect herbivore, compared with a non-mycorrhizal individual. Mycorrhizal effects on plants that could be of importance to insects include altered nutritional quality, chemistry or simple physical features such as overall size. Our knowledge of how mycorrhizas can affect phytophagous insects is restricted, but here I will give an outline of the interactions that have been found and will attempt to produce a synthesis of these patterns. I will address the mechanisms by which these interactions occur and will place the work in a multitrophic context by considering higher trophic levels as well.

7.14.4. Effects of belowground and aboveground organisms on plant floral traits and seed set

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In animal-pollinated plants, fitness is influenced by floral traits that function as advertisement and reward for pollinators. It is increasingly recognized that belowground and aboveground communities are highly interdependent and studies on how belowground organisms affect flowering traits and seed set may lead to a more integrative view of the functioning of terrestrial ecosystems. In a three-factorial common garden experiment we investigated single and combined effects of root herbivores, leaf herbivores and decomposers on floral traits and seed set of wild mustard (*Sinapis arvensis*). Reduced nutrient availability through leaf herbivory affected primarily floral traits that could be important for pollinator attraction. Improved nutrient supply through decomposer activity was invested in seed production but did not affect floral traits. Our results suggest that *S. arvensis* faced no strong selection pressure from pollen limitation. Ultimately, this study may lead to a more detailed understanding of how belowground and aboveground plant-animal interactions shaped the evolution of plant species.

7.14.5. Linking above and belowground multitrophic communities: consequences for diversity and ecosystem functioning

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Aboveground and soil dwelling communities are spatially separated. Plants exist both aboveground and in the soil and as primary producers they obviously influence both compartments. Evidence is now accumulating that organisms living above- and belowground can also influence each other via the plant. Most of this evidence is based on single interactions using one host plant and one aboveground and belowground organism. Using artificial grassland communities consisting of plant, soil, and aboveground communities, we will show how in a complex system above- and belowground communities can influence each others dynamics. Soil community composition influenced plant diversity and ecosystem functioning. Moreover, aboveground plant primary and secondary chemistry changed depending on soil community composition, resulting in reduced herbivore populations, but increased performance of the natural enemy of the herbivore, leading to an aboveground trophic cascade mediated by belowground organisms.

7.14.6. Nutrient competition may cause spatially structured herbivory on lupines at Mount St. Helens.

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A 3 yr nutrient addition experiment on primary successional substrates at Mount St. Helens, USA, demonstrated N limitation of most community members, but P limitation of *Lupinus lepidus*, the N-fixing, dominant colonist. P addition increased the proportion of lupin seeds filled (yr 1) and % cover (yr 3). Forb % cover also increased by yr 3 in P plots, suggesting N-P co-limitation of the community. Removing neighbors around focal plants increased

lupin relative growth (RGR), survivorship, and the RGR response to P fertilization. *L. lepidus* is subject to intense herbivory by lepidopteran larvae in low density patches that is absent in the high density patches studied here. Larvae raised in captivity typically have higher RGR on leaves from low density patches, which are usually higher in N and P. However, larval RGR and gross growth efficiency increased with leaf P content but not leaf N. In glasshouse, P fertilization increased leaf N and P and larval RGR. In the field, larvae placed on plants 1 yr after neighbor removal consumed more leaf surface area. We hypothesize that P limitation of lupin under competitive conditions excludes herbivores from high density patches.

7.14.7. Above- and belowground pathogens: driving the evolution of plant sexual reproduction?

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A popular but largely unproven hypothesis on why sexual reproduction is so widespread is that the production of variable offspring provides better protection against rapidly evolving pathogens. Scarce empirical tests of this hypothesis in plants have focused on the effects of aboveground pathogens. Increasing awareness that belowground pathogens can play an important role in shaping plant diversity prompts us to consider the potential role of soil pathogens. Do these pathogens pose selection pressures that are strong and specific enough to shape genetic variation within plant populations, and to drive the evolutionary maintenance of plant sexual reproduction? And how do their effects compare to or interact with those of aboveground pathogens attacking the same plant species? I will discuss existing views on this, and will outline a strategy to address the issue empirically using dandelions as a model system - a species that has sexual as well as asexual forms and that is attacked by both aboveground and belowground pathogens.

7.15.1. Human impact, ecosystem management and ecosystem conservation in protected areas

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The earth has always been under transformation by natural forces, but today it is being transformed by human action. The human imprint on the earth's landscape is profound: natural ecosystems are being transformed into highly altered systems. Even in protected areas, some ecosystems have been seriously degraded or destroyed. The consequences are: loss of biodiversity at all levels of organization, habitat destruction, extinction and endangerment of species. This is particularly serious in areas with a long history of intense use by humans and at the same time with high level of biodiversity. Among these areas those located in Mediterranean region are very significant. Here there are many hot spots of biodiversity. We urge that, particularly on protected areas, ecosystem management be conservative. In this areas the core of ecosystem management should be ecosystem protection and restoration. Therefore, where it is necessary to use natural resources we must carry out ecosystem management strategies which ensure to maintain their ecological integrity. Sustainable resource use is necessary to ensure the future of life on the earth.

7.15.2. National Parks Kopaonik and Tara (Serbia) - management and nature conservation challenge

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Two out of five National Parks in Serbia, Tara and Kopaonik Mts were enounced in the same year and are placed into the same law category, but they did not have the same destiny. The long-term negative influence on Kopaonik culminated in building of a huge hotel complex and ski paths at the ecologically most important and floristically richest area of the mountain. Consequences of direct and indirect negative influence, destruction, degradation and change of natural structure of phytocenoses led to serious disturbance of stability of ecosystems. The only chance for stopping this process and recovery of the area is the strict application of ecological principles and extremely sustainable use of natural resources on Kopaonik. On Tara Mt, due to controlled use of resources, there are some spacious very well preserved forest complexes, the main asset of which are the relict biocenoses and species. The state of ecosystems, conservation

challenges and possibilities of sustainable development in both National Parks will be discussed.

7.15.3. The importance of eco-coenotic conditions to the conservation of threatened plant populations from the natural reserves of Transylvania (Romania)

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Following a general presentation of the conservation strategies in Romania, we focus on the influence of eco-coenotic conditions on the structure of some threatened taxa populations distributed in Transylvanian protected areas. The studies performed on 4 taxa revealed significant differences with implication in their survival and *in situ* conservation: - the mean density, height and number of fertile shoots/*Astragalus peterfii* ramet are larger in *Stipa*-dominated open communities than in closed grasslands; - the number of flowers/*Trollius europaeus* individual is larger in sedge communities invaded by *Phragmites* compared with those free of reed, probably because of eutrophication; - *Dianthus petraeus* ssp. *orbilicus* has higher values of fertile shoots, number of flowers/shoot and shoot density/unit of basal leaf area in *Thymetum comosi* than in basophilous grasslands, probably because of a low competition in the pioneer community; - *Aquilegia nigricans* ssp. *subscaposa* has a larger morphometric variance in the oak-beech forest than in the adjacent grassland, most likely because the increased spatial/temporal heterogeneity of the forest understory.

7.15.4. Origin, present and future of the flora and vegetation in Pannonian protected areas

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Main values of the Pannonian flora and vegetation are protected in 10 National Parks, Land Protection Areas and more than 700 nature conservation areas in ca. 10 % of the national territory of Hungary. Their origin has been delineated in the Ősmátra (Ancient-Mátra) Theory put into new light in recent palynological, taxonomic, floristic and ecological evidences stimulating to arise a new vision, create a new scenario consisting of several thesis concerning the late glacial and Holocene evolution of the Pannonian flora and vegetation. A new interpretation of paleoendemics, holoendemics, schizo- and neoendemics has been elaborated for the region, delineating new relic areas for protection and proposing new areas for botanical research including tasks for the national nature conservation authorities. The parallel evolution of the Pannonian and the Ukrainian-Russian steppes, different models of evolution of the mountain and lowland endemics, the wooded vs. treeless character of the Hungarian lowland, the primer vs. secondary origin of the alkali vegetation and the main values of the flora and vegetation, their threat and conservation are also discussed.

7.15.5. Conservation and management of phytocoenoses in protected areas (central europe)

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The conservation of phytocoenoses is one of the most difficult environmental problems of our time. Phytocoenosis alterations involve changes to species composition and structure due to processes of degeneration: introduction of exotic species, disappearance of characteristic species, and simplification of the structure; those that provoke the disappearance of the phytocoenosis are regression, fragmentation, reduction and elimination. Theoretically, it is easier to conserve stable primary (zonal) phytocoenoses, which are self-governed by the process of fluctuation (such as forests) and hence only undergo natural disturbances; more complex is the conservation of secondary phytocoenoses (meadows, etc.) inasmuch as they undergo anthropic disturbances, and thus are not stable. The stable and azonal phytocoenoses (such as marshy associations) can be conserved only if the ecosystems are maintained in their entirety. Phytocoenosis conservation is of first priority in the protected areas, where man should intervene as little as possible in the

primary phytocoenoses, and adopt particular interventions of management in the secondary and azonal ones.

7.15.6. Vegetation-ecological management and restoration in and around protected areas in Japan

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In proportion to rapid nature destruction and urbanization seen in these 50 years, natural areas ranging from large natural parks to small shrine groves have been protected for the preservation of natural/ semi-natural ecosystem, biodiversity and their specific landscapes. Only preservation and management in protected areas, however, is not enough to deal with today's global nature devastation. It is mandatory to restore positive green environments, especially indigenous forests, in our living areas. Since the 1960s, we have been conducting field investigations of all kinds of vegetation including that of nature reserves. We identify the potential natural vegetation through research and keep planting tree species from it, for the restoration of quasi-natural ecosystems. We regenerated green environments at about 1500 sites including 1200 sites in Japan as well as in Borneo and China. Our tree-planting activity is future-oriented, proactive nature preservation, not mere maintenance and elucidation within limited protected areas. This is a mission for human existence that field botanists of all over the world have to carry out in their own regions.

7.15.7. Primary succession on barren substrates: lessons for restoration of protected lands

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Primary succession requires amelioration, dispersal, establishment, and development. Biotic interactions, landscape effects, changing safe-site qualities, and chance all affect succession. Studies on many volcanoes (e.g. Etna, Fuji, St. Helens, Tolbachik, Tarawera, Kilauea) reveal lessons to improve restoration of damaged habitats within protected areas. Trajectories develop at different rates due to stress and in different ways due to priority effects and dispersal limitations, producing natural mosaics. Recovery can be hastened by alleviating stress through appropriate fertilization. Very short distances limit dispersal of most species, so managers must continually facilitated dispersal. Competition and herbivory can slow succession, so active management may be needed. Alien species may be controlled by biotic factors. Natural processes can produce several alternative, stable, natural plant communities, so a mosaic of vegetation in protected natural areas should be encouraged. Monitoring through permanent plots is required to detect invasions by alien species and responses to climatic changes.

7.16.1. Kawa Kharpo and Kinabalu: Comparative alpine ethnobotany in the Subtropics and Tropics

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Kawa Kharpo (6740m) is a Tibetan sacred peak in the eastern Himalayas; *Kinabalu* (4101m) is a Dusun sacred peak in Borneo. The flora, use and meaning of these two mountains differ dramatically and yet have striking similarities. Both have steep elevational and corresponding vegetational gradients, however in the tropics biodiversity declines predictably with elevation, while in the Himalayas the highest biodiversity is found in Alpine Meadows. In both areas useful plants, predominantly medicinals, are directly correlated with biodiversity. Furthermore, numerous plant resources are managed on *Kawa Kharpo*: Tibetans collect mushrooms, pasture yaks, grow crops, offer religious plants, and nurture famous Himalayan horticultural plants - lilacs, peonies, primrose, fritillaries, rhododendrons. For both ethnic groups, conservation is traditionally sacred, but recently they must address tourism, commercial interests, and government policy. Global warming affects both massives, pushing threatened alpine flora - including valuable medicines - toward extinction. Alpine ecology and culture - Ethnoecology - account for much similarity and difference.

7.16.2. Alpine ethnobotany in the Caucasus and Central Asia

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More than 7,000 species of economic plants are listed for the former Soviet Union, mainly from two botanically rich mountain regions: the Caucasus and Central Asia. Above timberline, there are about 90 useful spp in the Caucasus and about 150 in Central Asia (Tien Shan, Pamiro Alay). These Alpine areas are threatened by habitat loss to development and overgrazing. The Caucasus and Central Asia have distinct flora with few species in common; cultural differences are likewise great and ancient. Some species present throughout the areas are used for different purposes (e.g. *Paeonia* sp. by Georgians and Kazkhs; *Gentiana schistocalyx* by Adjars and Abkhazs), while different but related species are used for the same purposes (*Valeriana daghestanica*, Caucasus by Daghetanians and Ossetians, *V. fedtshenkoi*, Central Asia by Kirgyzs). Limited supplies of useful plants at lower elevation have forced people to use alpine plants for new purposes (e.g. *Comarum salesovianum* as a tea, in Pamir by Tajiks; *Berberis sphaerocarpa* for vine production, in Central Asia by Kazakhs). These high mountain vegetations are endowed with new species, new uses and new directions in Ethnobotany.

7.16.3. Unconventional wild plant and insect consumption in the southern Alps of Friuli, Italy

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Folk diets include wild plants and a few invertebrates in Friuli that are peculiar in the Alps. Recovery of scattered knowledge is valuable for maintaining use and enhancing a base for agroecology, conservation and ecotourism. *Pistic*, *Frita*, *Litum* are common names from about 80 herbs collected in field margins, hay meadows, woodlands and the wild, most commonly in spring. To eat, most herbs are boiled and later sauteed with butter or lard and garlic. In the higher range traditional alpine slopes have supported seasonal sheep and cow grazing. Summer pastoral communities have traditionally used plants for salads, soups, spices and cheese production. Collections of these useful alpine plants include: *Chenopodium bonus-henricus*, *Aruncus dioicus*, *Cicerbita alpina*, *Rumex pseudoalpinus*, *Carlina acaulis*, *Myrris odorata*. Some insects are also eaten in these meadow areas such as adult Lepidoptera (Zygaenidae and Ctenuchidae). These local traditional uses need more ethnobiological attention and study.

7.16.4. At the edge of the tree line: Ethnobotanical importance of high elevation sites in the Northwestern North American interior region

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The Coast and Cascade mountains of northwestern North America, from central British Columbia to northern California, have immense ethnobotanical significance, with well over 50 culturally important plant species documented. For millennia, indigenous peoples living in the valleys below have been moving up to the high country in the summer months, camping at the tree line, hunting and harvesting diverse plant resources, including foods (e.g., *Vaccinium membranaceum*, *Claytonia lanceolata*, *Erythronium grandiflorum*, *Pinus albicaulis*), medicines (e.g., *Veratrum eschscholtzii*, *Valeriana sitchensis*) and materials such as fuel. Upland plant communities have been traditionally managed in myriad ways. These montane areas are essential traditional territory, and although still required for nutritional, educational, and cultural purposes, people's access to them has often been restricted. Their biodiversity and productivity have been threatened by mining, overgrazing, logging, invasive species, fire suppression, and even tourism. Yet, these areas are still critically important within the indigenous communities and the ecology of the entire region.

7.16.5. Crop and wild plant biodiversity in the Andes: traditional management, threats, and conservation in highland communities

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All alpine regions have elevational gradients, but the Andes' unique shape and location provide a moisture gradient between the dry western flank and the cloud forests of the eastern slopes, as well as a long latitudinal gradient with a range of seasonality and photoperiod cues. This ecological heterogeneity fosters extreme endemism in the tropical montane regions. The Central Andes are unparalleled among alpine regions as a center of crop domestication and agrobiodiversity. Traditional management of agroecosystems and crop diversity includes terracing, burning high pasturelands, multicropping, cultivating diverse homegardens, and community-level sectoral crop rotation/fallowing. Andean heterogeneity means that threats to biodiversity vary across the region: crop diversity is threatened by overpopulation in some areas and depopulation in others, deforestation threatens wild crop relatives, mining pollutes rivers, and the future effects of global climate change are yet unknown. How exchange of propagation material affects the genetic structure of clonal crop populations is currently under study to aid both old and new methods of conservation.

7.17.1. Golden Rice: Genetic engineering technology against malnutrition.

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Vitamin A-malnutrition is responsible for ca 6'000 death per day, and leads to 500'000 blind children per year. Biofortification could lead to sustainable and cost-effective solutions. 'Golden Rice' contains transgenes which lead to synthesis and accumulation of provitamin A. Since the proof-of-concept publication (Science 2000) the provitamin A-content has been increased to ca. 20-fold above that publication. A typical daily diet of rice can prevent VADD if based on Golden Rice. The trait has been transferred to and is functioning in more than 30 popular rice varieties. The first field release experiment did not indicate any agronomic problem, a professional taste trial was positive, and no ecologist can propose a substantial risk to any environment. Free licences for humanitarian projects for IPR involved allow for use by small-scale farmers and traders without any costs, there is no additional agronomic input required, and the farmer can use part of his/her harvest for the next sowing. Golden Rice could save numerous children from blindness and death but extreme precautionary regulation and radical GMO opposition are preventing this, so far.

7.17.2. Production of natural products by plant cell biotechnology

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In 1959 Tulecke and Nickel were the first to report the mass cultivation of plant cells in some hundred liters with the intention to produce natural products. More than 20 years later in 1982 Mitsui Petrochemical Company in Japan established the first commercial process of natural product (shikonin) formation by plant cell cultures. Whereas shikonin is produced on a rather small scale, in 2004 Phyton Deutschland GmbH in Ahrensburg (Germany) announced a real breakthrough, the production of the anticancer compound paclitaxel by *Taxus* cell suspension cultures in a 70 m³ (working volume 50 m³) bioreactor which is used by Bristol-Myers Squibb for Taxol[®] production. (press release by Phyton in 2004). The reasons why it took so long time before the dream of the large scale commercial production of a medicinal compound became true will be discussed. Additionally however, the numerous possibilities still offered by plant cell biotechnology concerning natural product formation will be described using lignan accumulation in *Linum* cell cultures.

7.17.3. Biotechnological applications for the improvement of grapevines

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Plant biotechnology as an interdisciplinary science is able to provide solutions to agricultural challenges, particularly in the case of woody fruit crops, e.g. by rapid propagation of selected cultivars, conservation of valuable germplasm, phytosanitary and genetic improvement and safeguarding human health not only through

nutritional, but also through ecological aspects. Data obtained from the molecular characterisation of 5 Portuguese *Vitis vinifera* ssp. *sylvestris* indicate that wild grapevines had a major influence on the domestication of cultivars. From a greenhouse collection of *V. vinifera sylvestris*, accessions were introduced in vitro as a conservation strategy and to obtain quickly propagation material under axenic conditions to be used for studies to identify genotypes with putative resistance to the fungi *Plasmopara viticola* and *Uncinula necator*.

7.17.4. Somatic embryogenesis - the gateway to biotechnology in conifers

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Clonal propagation by conventional techniques of most of the conifer species used as forest or Christmas trees is difficult or inefficient. Biotechniques, primarily somatic embryogenesis (SE), provide a powerful alternative for establishment of clonal varieties and shortening of breeding programs. In gymnosperms, somatic embryogenesis has been demonstrated for the first time in 1985 and, up to now, shown in more than 50 species and hybrids. In the recent years, integration of SE into breeding strategies and commercialisation of SE based propagation has been started. Additionally, embryogenic cultures are an ideal target for Agrobacterium-mediated and direct gene transfer as well as fundamental studies in embryology. In the presentation fundamentals of SE in conifers, present achievements in practical application and limitations will be discussed based on own results in *Larix*, *Abies* and *Pseudotsuga* species.

7.17.5. Differences and common features of developing somatic and zygotic embryos of oak (*Quercus robur* L.)

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In several broad-leaved tree species, including also oak, somatic embryo (SE) maturation represents a major barrier to the commercialization of this vegetative propagation system. To improve the quality and germination frequency of SEs, comparative biochemical and molecular studies were performed to study the physiological changes during zygotic and SE development. The water status, proline, starch, endogenous ABA and IAA levels of the developing embryos were monitored in relation to morphological characters. The temporal and tissue specific expression patterns of the *legumin* gene, the major storage protein of oak, and several oak *lea* genes (late embryogenesis abundant proteins) were investigated. Differential expression patterns of some *lea* genes were found, whereas the expression in zygotic and somatic embryos of other *lea* genes is embryo specific and developmentally regulated. Variations in phytohormone levels in combination with changes in tissue water status seem to be important factors for both zygotic and somatic embryo development.

7.17.6. In vitro collection and sanitation of valuable germplasm of temperate fruit trees

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Conservation and utilization of biodiversity in a sustainable manner represent major priorities. To conserve genetic resources in different type of genebanks (*in vitro*, *in situ*, *ex situ*), genetic markers may support an informed choice of genotypes, e.g. molecular markers like RFLP; AFLP and SSRs. Improved strategies for the production of elite plants of pathogen-free stone fruit cultivars include on one hand the application of rapid, reliable, user-friendly, sensitive and cost effective methods for the detection and elimination of the major stone fruit tree viruses and phytoplasmas and on the other hand the elaboration of molecular markers for genotype characterisation. An *in vitro* collection of apricots and peaches containing cultivars testing positive for Plum Pox Virus (PPV) and European Stone Fruit Yellows (ESFY), was established and used as model system for virus and phytoplasma elimination. Different *in vitro* techniques, i.e. meristem culture and heat therapy *in vitro* were applied either alone or in combination and results compared. Protocols were validated for high survival rates of plants and for their effectiveness for pathogen elimination.

7.17.7. In vitro culture for propagation and breeding of ornamental plants

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In vitro culture techniques are commercially used for clonal propagation of high value crops, among which many ornamental species are found. The statistics of German commercial tissue culture labs clearly demonstrate the special impact of micropropagation for orchids, mainly in the genus *Phalaenopsis*. Since the high input of manual labour results in high prices, part of the production is transferred to low wage countries. An alternative approach is automation, for which liquid culture systems are particularly suited. *In vitro* culture techniques are not only applied for mass propagation of high priced species in floriculture, but we cannot imagine multiplication of vegetatively propagated ornamentals without virus elimination by meristem culture and clean nuclear stocks. For commercial micropropagation, axillary branching is the method of choice for the vast majority of species. Regeneration via organogenesis and somatic embryogenesis plays only a minor role, but is very important for the production of transgenic ornamentals. The applications of somatic embryogenesis for propagation and breeding of *Cyclamen persicum* will be given as an example.

8.1.1. Specificity in auxin signalling

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Auxin regulates transcription by reducing the stability of Aux/IAA transcriptional repressor proteins. Aux/IAAs repress transcription by dimerising with members of the ARF family of DNA binding proteins. Degradation of the Aux/IAAs destroys the dimers allowing activation of transcription. Furthermore, Aux/IAA proteins can dimerise within the family. Such Aux/IAA-Aux/IAA dimers could regulate the formation of ARF-Aux/IAA dimers by competition. There are 29 Aux/IAAs and 23 ARFs in the *Arabidopsis* genome, and thus a large number of possible dimers. This suggests a network in which diverse, tissue and dose-dependent auxin responses are encoded by tissue-specific expression, and changes in the relative abundance of Aux/IAA-ARF network components. To test this model we are using stabilised Aux/IAA mutants. Strikingly, such mutants can have entirely opposite phenotypes. For example, stabilised BDL/IAA12 produces highly bushy plants, but similar mutations in AXR3/IAA17 confer reduced shoot branching. To understand how such functional specificity is encoded, we are using promoter swapping, domain swapping, and mis-expression experiments.

8.1.2. N-terminus of a rice ABRE binding factor controls transcriptional activation function by protein phosphorylation

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Abscisic acid (ABA) mediates responses of a plant to environmental stresses in which diverse protein kinases and transcription factors are involved. Here, we characterized a rice ABA-response element binding factor (OREB1) previously identified as an *in vitro* substrate of a rice SnRK2 kinase. OREB1 has transcriptional activation activity in yeast and deletion of N-terminus of 92 amino acid resulted in a complete loss of the activity. Sub-deletion analysis showed that both positive and negative regulatory domains are present in the N-terminus region and amino acid from 42 to 119 showed the possibility to act repressive function. MALDI-TOF MS analysis of the *in vitro* phosphorylated OREB1 revealed a site of phosphorylation to ser-44 by a rice SnRK2 kinase. Indeed, a triple mutant of serine-43, 44, 47 to alanine resulted in an increase of transcriptional activation activity in yeast, implicating that these phosphorylation sites are important to control the repressive function. Our present findings indicate that SnRK2-mediated phosphorylation of the N-terminus of OREB1 is an important mechanism to regulate its function, possibly, in the negative regulation.

8.1.3. Cytokinin signaling regulates vascular morphogenesis

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Cytokinin regulates several aspects of plant development and physiology. Our lab is studying the genetic control of vascular morphogenesis during root development in *Arabidopsis*. A recessive mutation, *wooden leg (wol)*, in the *CRE1/WOL/AHK4* gene coding for a cytokinin receptor, results in reduced cell proliferation and exclusive xylem differentiation (Mähoenen *et al.* *Genes&Dev.* 14, 2938-2943; Inoue *et al.* *Nature* 409, 1060-1063). This indicates the involvement of a specific cytokinin mediated morphogenetic pathway during early stages of vascular development. In order to more systematically approach the genetic control of cell proliferation during vascular development, we have carried out an EMS based screen for suppressors of *wol* based on root growth pattern. Both extra- and intragenic suppressors were identified. The presentation will focus on the further characterization of these mutations.

8.1.4. The transcription factor FUSCA3 controls developmental timing in *Arabidopsis* through the action of multiple hormones

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Morphological differences among closely related species can often be explained by shifts in the relative timing of developmental programs. Understanding the molecular basis of what regulates these shifts in plants and animals has led developmental biologists to identify genes that regulate the timing of appearance of various organs and structures through an organism's lifespan. In *Arabidopsis*, loss-of-function (lof) mutations in the *FUS3* gene cause embryonic leaves to take on more adult fates while constitutive misexpression produce cotyledon-like leaves during vegetative development. We find that the timing of *FUS3* expression specifies leaf identities by controlling the duration of the foliar organ program. One mechanism by which *FUS3* control foliar organ identity in *Arabidopsis* is by regulating the synthesis of two hormones, abscisic acid and gibberellin. These hormones in turn, regulate the rates of cell cycling during organ formation to determine whether an embryonic or adult leaf will emerge. *FUS3* expression is also dependent on the distribution of the patterning hormone, auxin, and therefore acts as a nexus of hormone action during embryogenesis.

8.2.1. Protein import into chloroplasts

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Chloroplasts are of endosymbiotic origin. During this process most of genes were transferred from the endosymbiot to the arising host nucleus. Therefore chloroplasts have to import between 3000 and 4000 different polypeptides. These proteins are synthesized in the cytosol as precursor proteins, which contain in most cases an N-terminal targeting signal, also called transit peptide. The transit peptide is recognized at the chloroplast surface and translocation is initiated. This task is accomplished by two distinct translocons localized in the inner and outer envelope in a GTP and ATP dependent manner. In the stroma the transit peptide is cleaved. Protein import is regulated by phosphorylation and GTP on the cytosol / chloroplast interface and by redox control as well as calcium at the stroma / inner envelope interface. Furthermore, the composition of the protein import translocon depends on developmental status of the organelle as well as the plant organ. Therefore chloroplast protein import is highly regulated at the molecular and cellular level.

8.2.2. Chloroplast protein import in *Arabidopsis thaliana*

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In *Arabidopsis*, a small family of six Toc-GTPases, consisting of a subgroup of four large proteins (atToc159/132/120 /90) and two smaller ones (atToc34/33), controls access of precursor proteins to the chloroplast. The Toc-GTPases are organized in Toc-complexes. The minimal Toc-complex is considered trimeric. It consists of one large and one small Toc-GTPase as well as atToc75, a protein-conducting channel. Mutant analysis of the Toc-GTPases has led to the operational definition of two classes of precursors: photosynthetic proteins relying on Toc159/90/33 for import and housekeeping proteins relying on Toc132/120/34. Moreover, the existence of corresponding Toc-complexes involving the different sets of Toc-GTPases has been demonstrated. Biochemical studies indicate that the GTP-binding domains of Toc-

GTPases play a key role in the assembly of Toc-complexes. Here, we present data on the role of atToc90 in the import of photosynthetic precursor proteins. In a second part, we will detail the role of specific amino acid residues and guanine nucleotides in the assembly of atToc33 and -159.

8.2.3. Protein targeting into "complex" diatom plastids: Analysis of a new type of targeting motif.

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Secondary endocytobioses, i.e. the incorporation of a photoautotrophic eukaryotic cell by a eukaryotic host cell and the subsequent transformation into a plastid, dramatically enhanced the complexity of the resulting chimeric cells. As a result diatom plastids have four envelope membranes, the outermost being continuous with the endoplasmic reticulum („chloroplast ER"/"CER"). *In vivo*-analyses of targeting of fusion proteins consisting of presequence domains fused to GFP indicate that protein import into diatom plastids involves at least two steps: a cotranslational transport step at the CER or the cytosolic ER and a further import step resembling protein import into plastids of higher plants. Utilizing deletion constructs we found accumulation of the GFP fusion proteins in a compartment that apparently is attached to the envelope membranes of the plastids, having a „blob"-like or reticular structure. Comparison of different presequences revealed a so-far unknown conserved "AFAP" or "ASAF" motif at the N-terminus of the transit peptide. Site directed mutagenesis revealed that the phenylalanine residue is essential for successful protein import.

8.2.4. Targeting and import of peroxisome membrane proteins

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Peroxisomes perform a wide range of vitally important functions in plant cells. In addition to well established roles in mobilisation of seed storage reserves and photorespiration new functions have recently emerged. These include formation and turnover of the major signalling molecules, NO and H₂O₂, and the biosynthesis of IAA (from IBA) and Jasmonic acid (from OPDA). Many of the functions of peroxisomes are mediated by membrane proteins, and membrane proteins play essential roles in the import of all the other matrix located proteins of peroxisomes. Our knowledge of peroxisome membrane protein targeting and insertion is rather incomplete. For example whether some peroxisome membrane proteins traffic to peroxisomes from the endoplasmic reticulum is still a matter of debate, as is the role of potential receptor/chaperone molecules. Our studies indicate that two peroxisome membrane proteins, AtPEX10 and AtPEX2 are not trafficked to peroxisomes via the ER. AtPEX10 interacts with the membrane protein chaperone AtPEX19 which is proposed to bind peroxisome membrane proteins in the cytoplasm and chaperone them to the peroxisome membrane.

8.2.5. Nucleo-cytoplasmic partitioning of transcription factors: a tool for the regulation of signalling.

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The separation of transcription and translation by the nuclear envelope offers an additional level to control gene expression in eukaryotic cells that is not available in prokaryotes. Regulated nucleo-cytoplasmic partitioning of transcription factors can be achieved by controlling nuclear import, nuclear export, and/or degradation. In *Arabidopsis thaliana*, we characterised the nuclear export pathway that depends on the nuclear export receptor exportin 1. Identifying a nuclear export signal in a transcription factor strongly suggests a role of nuclear export in the regulation of gene expression. As a novel approach to plant signal transduction we screened for *Arabidopsis* transcription factors that contain nuclear export signals. Many different transcription factors that belong to different families were identified in this way. Our results indicate that nuclear export is an important tool to regulate diverse signalling pathways in plants.

8.2.6. Exploring plastid division pathways in *Arabidopsis*

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Plastids arise by division and the control of division site placement is mediated in part by AtMinD1 and AtMinE1 in *Arabidopsis*. We have shown that AtMinE1 is a topological specificity factor and that AtMinE1 forms homodimers and heterodimers with AtMinD1. AtMinE1 and AtMinD1 show specific intraplastidic localisation patterns and a C-terminal helix deletion in AtMinD1 results in inappropriate localisation and disrupted plastid division. Using FRET we have shown that AtMinD1 is capable of forming homodimers inside chloroplasts and that this dimerisation capacity is abolished by a single C-terminal A296G mutation. Our data demonstrates that AtMinE1 and AtMinD1 act in concert and that the formation of AtMinD1 homodimers and AtMinD1/AtMinE1 heterodimers is paramount for correct intraplastidic localisation patterns and correct division machinery placement. The assembly of the different AtFtsZ proteins in chloroplasts in relation to the AtMinD1/AtMinE1 protein complex will also be discussed. GIANT CHLOROPLAST 1, a positive factor involved in plastid division will be briefly described. A working model of plastid division will be presented.

8.2.7. Friendly: a mitochondrial dynamics mutant defective in mitochondrial inheritance?

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Mitochondria are ubiquitous and vital eukaryotic organelles. Although identified over 50 years ago as the site of oxidative energy metabolism little is known about the genetic control of mitochondrial shape, size, number and distribution (collectively termed mitochondrial dynamics) in higher plants. We identified a suite of *Arabidopsis* mutants with altered mitochondrial dynamics and are using these as tools to identify the underlying genes, proteins and mechanisms. In one mutant, *friendly* (*fmt*), mitochondria form clusters of tens of organelles. The *fmt* locus was identified and the gene, *FMT*, is homologous to the *D. discoideum* *cluA* and *S. cerevisiae* *CLU1* genes that are known to be involved in the maintenance of the correct cellular distribution of mitochondria. Apart from a tetratricopeptide repeat domain, the FMT protein has no significant homology to proteins of known function. We will present data testing the hypotheses that: (i) FMT is involved in association of mitochondria with the cytoskeleton and (ii) the clustered mitochondrial phenotype in *friendly* perturbs the stochastic inheritance of mitochondria into daughter cells during cell division.

8.3.1. Specifying the site of tip growth and the plane of asymmetric division in fucoid algae

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Eggs of fucoid algae are initially apolar cells and fertilization initiates a developmental program that breaks the symmetry resulting in selection of a site of localized tip growth. Growth site selection, which is influenced by a variety of environmental vectors, involves assembly of a cortical actin network nucleated by an Arp2/3 complex at the presumptive growth site. This nascent axis is labile and perception of subsequent spatial cues results in disassembly of the existing cortical actin and reassembly at the newly established growth site. Once tip growth has been initiated, the growth axis positions the mitotic spindle, which in turn determines the plane of asymmetric division. The spindle is positioned by centrosomal microtubules that interact with the cell cortex preferentially at the growth site, perhaps mediated by the microtubule-associated protein EB1. We anticipate that identification of additional molecules involved in signal transduction, actin assembly and microtubule search and capture will be facilitated by ongoing genomics projects.

8.3.2. Immediate upright (*imm*), a mutant of the marine macroalga *Ectocarpus siliculosus* (Phaeophyceae, Ectocarpales) affected in polarity and development

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Sporophyte and gametophyte are independent generations in *Ectocarpus siliculosus*. Wild-type sporophytes show bilateral

symmetric germination resulting in the development of a prostrate, branched filament. The first germ tube is positively phototropic in response to unilateral white light. Erect thalli develop by 'mediate differentiation' i.e. they do not form before the prostrate thallus has reached a certain size. Gametophytes, in contrast, exhibit a developmentally asymmetric bilateral germination and 'immediate differentiation'. The first germ tube is negatively phototropic and develops into a thin rhizoid, whereas the second germ tube immediately develops into a broader and erect (positively phototropic) filament. We describe a spontaneous mutant *immediate upright* in which the sporophyte has a germination pattern resembling that of gametophytes, including negative phototropism of the first germ tube and immediate development of an upright filament. Crossing studies showed that the mutation is recessive compared to the wt and is located on an autosome. This is the first genetic study of a developmental mutant in *Ectocarpus*.

8.3.3. Signalling, actin, endosomes and root hair tip growth

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Root hair tip-growth is actin- and signalling-dependent process. Both endogenous and YFP-tagged SIMK, a stress-induced mitogen-activated protein kinase (MAPK), accumulate in vesicle-rich tip regions of *Medicago* and *Arabidopsis* root hairs. SIMK distribution and function correlates with the organization of the actin cytoskeleton visualized by immunolabelling and with new F-actin *in vivo* marker GFP-ABD2. MAPK inhibitor UO126 abolished tip growth by disrupting vesicular traffic but overexpression of constitutively active SIMK overcomes this growth inhibition, suggesting a role of SIMK in actin-dependent vesicular traffic. Overexpression of SIMK activator SIMKK in *Arabidopsis* resulted in overactivation of AMPK6 (SIMK homologue) and phenotype of ectopic and branched root hairs. Additionally, several MAPKs associate with endomembranes and endosomes in control and stressed cells. New endosomal molecular markers FYVE and RabF2a were developed and will be presented as useful tool for studies on actin-dependent motility of endosomes in root hairs.

8.3.4. Microtubules in *Medicago truncatula* root hairs during hair development and response to rhizobial signal molecules.

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Tip-growing legume root hairs have two populations of microtubules: cortical microtubules (CMTs), which are present in all stages of root hair development, and - differently from *Arabidopsis* root hairs - a dense array of endoplasmic microtubules (EMTs), which the hairs acquire during the initiation of tip-growth and maintain until growth stops. EMTs are crucial to the polar distribution of cytoplasm, to nuclear positioning, and to a high growth rate.

In tip-growing hairs, signal molecules of symbiotic rhizobacteria (Nod factors) cause a subtle and transient shortening of the EMT array, whereas in growth-arresting hairs this effect is more pronounced and leads to a complete disintegration of the EMT array. The latter have a short EMT array and respond to Nod factors with a distinct change in hair morphology, called root hair deformation. The Nod-factor induced disappearance of EMTs correlates with a loss of polar cytoarchitecture and straight growth directionality, whereas the reappearance of EMTs correlates with the new set up of polar cytoarchitecture and new growth potential in growth-arresting hairs. CMTs showed no obvious response to Nod factors.

8.3.5. Vacuole form and movement in growing root hairs.

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Tip-growing root hairs are useful models of polarized plant cell growth. Root hair vacuoles must expand rapidly as hair tips grow to maintain turgor pressure through water uptake. Vacuolar

biogenesis in plant cells is a complex and variable process. We are using growing root hairs of the model plant *Arabidopsis thaliana*, plus ER or vacuole specific dyes and targeted green fluorescent proteins, to examine the form, distribution, movements, and expansion of root hair vacuoles. Vacuoles are labeled with the fluorescent pH indicator BCECF by growing seedling roots in a low concentration of the dye, which has no significant effect on hair growth. In wild type hairs, labile tubular elements of the vacuole extend into the cytoplasm-filled subapical regions. In *rhd3-1* mutants (which have a defective secretory pathway and altered ER morphology), vacuole morphology is also altered as spherical elements appear near the hair tips. This morphology may contribute to the defective control of tip growth direction that is characteristic of the mutant hairs.

8.3.6. The role of the cytoskeleton in the intracellular accommodation of microsymbionts by plant cells

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Two groups of plants can enter root nodule symbioses with nitrogen-fixing soil bacteria. Gram-negative, unicellular rhizobia induce nodules on legume roots, while Gram-positive mycelial actinomycetes of the genus *Frankia* induce nodules on the roots of plants from eight angiosperm families, mostly woody shrubs, collectively called actinorhizal plants. Based on molecular phylogenetic studies, both types of root nodule symbioses are supposed to go back to a common ancestor. We compared infection thread growth in actinorhizal and legume nodules by immunolocalization of components of the cytoskeleton. We used myosin VIII-specific antibodies and antibodies against actin and tubulin to analyse the intracellular accommodation of microsymbionts in legume (*Medicago truncatula*, pea) and actinorhizal symbioses (*Casuarina glauca*, *Datisca glomerata*) on light microscopy and TEM level. Myosin VIII accumulated around infection threads in actinorhizal systems, but not around legume infection threads. This study was supported by the Russian Foundation for Basic Research (04-04-48282) and by the German Research Council (DFG).

8.3.7. Biophysics of tip growth in fungi

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Much is known about the cytology and biochemistry of tip growth in fungal hyphae, but a fuller understanding requires knowledge of physical aspects, namely the spatial pattern of cell-wall expansion and the force expanding the wall. A 3-D mathematical model of hyphal morphogenesis was the basis to design and interpret experiments to elucidate the pattern of cell-wall expansion. The 3-D model was built under the assumption that polarized growth arises from a gradient of wall-building vesicles released by an advancing vesicle supply center (VSC). The VSC corresponds in position and function to the Spitzenkörper. We analyzed the trajectory of external and internal hyphal wall markers in *Rhizoctonia solani* and discovered that they followed orthogonal paths. Such finding supports the long-held view that turgor pressure, and not the cytoskeleton as recently suggested, drives cell wall expansion. The VSC model predicts that a polarized growth process with a decreasing coefficient of anisotropy generates the orthogonal pattern of wall expansion; this conclusion contradicts an earlier belief that tip growth in fungal hyphae is mainly an isotropic process.

8.4.1. Variation and similarities in pollen features in some basal angiosperms with some taxonomic implications

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An illustrated exploration is made of variation in pollen features within and between species and genera in several basal angiosperm groups including members of the Trimeniaceae (one species of which has dimorphic pollen), Winteraceae and Monimiaceae (*sensu lato*). Is there any variation in pollen morphology within a species, from one region to another? Stability in pollen features in a genus or species is not always reflected in

stability in other characters, such as floral structure and organ venation in these plants of ancient lineage. For example, no two stamens in a single flower of some species of Winteraceae have the same venation. On the other hand, the strictly dimerous nature of the unilocular gynoeceum of *Takhtajania*, so different from the carpels of the other members of the family, seems an enigma. These non-pollen features are discussed and illustrated too. The possible taxonomic implications of variation in pollen morphology within genera and possible links between pollen features and pollination ecology is noted.

8.4.2. Identification of fossil angiosperm pollen and its bearing on interpretation of the origin of angiosperms.

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Studies in the 1970's reporting the occurrence of fossil pollen types in the Cretaceous, coupled with surveys of extant pollen morphology of primitive flowering plants, laid the foundation for proposing a Lower Cretaceous origin of angiosperms. Over the last 30 years, morphological, ultrastructural, and ontogenetic studies of both extant and fossil pollen have provided an array of new characters and character states, as well as greater resolution in defining character polarities. Moreover, a range of fossil pollen types exhibiting angiosperm characters occur in low frequency within Triassic and Jurassic sediments. In this presentation we examine the use of pollen characters to track the origin and early evolution of angiosperms. Pollen data provide evidence for a pre-Cretaceous origin. For example, speciation and extinction rates were likely equal during the Triassic and Jurassic, resulting in the paucity of angiosperm pollen types from different geographic areas in the Atlantic rift zone. Not until the Lower Cretaceous did origination rates exceed extinction rates, resulting in the subsequent diversification of angiosperms.

8.4.3. Importance of pollen morphology for reconstructing plant evolutionary history: a comparison of Myristicaceae (Magnoliales) and Proteaceae (Proteales)

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The implications of a recent phylogenetic study of Magnoliales for pollen character evolution are presented, with an emphasis on Myristicaceae. In several instances, long-standing ideas on pollen evolution are completely turned upside down. In particular, columellar exine structure is unambiguously resolved as the ancestral state of Magnoliales whereas granular exine structure evolved later within the order, at least twice independently. Other characters, such as the shape of pollen grain in Myristicaceae, turn out to be too homoplastic to be given any phylogenetic significance. These results illustrate the crucial importance of studying pollen evolution in a broader phylogenetic context, using hypotheses derived from additional sources of data. This approach is critical to reconstruct the biogeographic history of Proteaceae (Proteales), with a specially rich pollen fossil record, whose assignment to modern taxa of the family needs complete revision to use the benefits of the cladistic approach and new data on phylogenetic relationships within Proteaceae.

8.4.4. Compound pollen formation in *Annona* and *Cymbopetalum* (Annonaceae)

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Anthers of *Annona* are septate and polysporangiate, each sporangium contains a single microsporocyte. Meiotic tetrads build up a well-developed proexine proximally but only a thin pronexine distally while enclosed in the callosic envelop. During callose digestion, due to the tight conjugation between the distal pronexine and the envelop and, probably, the presence of celluloses in the envelop, the four microspores move out of the chamber in rotation but are bound to a central, undigested callosic-cellulosic mass. The mass then transforms into the binding core of the tetrad pollen. In *Cymbopetalum* each sporangium contains two microsporocytes; abundant cytoplasmic bridges exist between them and the two soon developed callosic envelopes. While each microspore tetrad undergoes microspore rotation and the formation of a binding core as in *Annona*, the two binding cores in each sporangium integrate into a central one to keep the eight

microspores as one unit; the eight microspores soon distribute evenly and constitute a perfect octad.

8.4.5. The fossil history of Araceae pollen revisited

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Only few years ago the fossil pollen record of Araceae was extremely sparse, consisting of a single record dating from the Late Miocene (Spathiphyllum type). Recent findings of unequivocal Araceae pollen types have now changed the situation completely. Based on comparisons with extant taxa we found in various fossil assemblages distinctive Araceae pollen types with an elaborated sporopollenin ectexine. These types, namely a polylicate-inaperturate type (similar to the Spathiphyllum type), a zona-aperturate-foveolate type (similar to the Monstera/Gonatopus type), and a spiny-ulcerate pollen (representing the Limnobiophyllum/Pandaniidites type) are usually not rare in their respective assemblages, and sometimes the samples consisted of practically 100% of such a pollen type. The fossil history of this extant monocot family now extends into the late Early Cretaceous. By the Latest Cretaceous to the Paleocene and Eocene these Araceae pollen types are widespread and provide clear evidence that several Araceae lineages have developed since the Mid Cretaceous and became diverse in the Paleogene.

8.4.6. Exploding pollen

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Montrichardia arborescens (Araceae) is distributed in tropical America and West Indies. It grows in tranquil freshwater habitats, forming dense stands along river margins. Pollen grains of Montrichardia are inaperturate with psilate ornamentation. The pollen wall is formed by a thin ectexine and an extraordinary thick intine. Contact with water leads to a rapid swelling of the intine followed by an explosive opening of the exine. Within few seconds a thick tube is formed, which is not the pollen tube. At the tip of the tube the pollen protoplast is situated. So far, the function of the intine tube is unknown. Apparently it is not involved in pollination. Gibernau et al. (2003) report beetle-pollination for Montrichardia. Lit.: Gibernau M, Barbé D, Labat D, Cerdan P, Dejean A (2003) Reproductive biology of Montrichardia arborescens (Araceae) in French Guiana. J Tropical Ecol 19: 103-107.

8.4.7. Comparative structure and development of the pollen and tapetum in Pandanales.

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The newly recircumscribed monocot order Pandanales now includes five families: Cyperaceae, Pandanaceae, Stemonaceae, Triuridaceae and Velloziaceae. We present new data and a review of pollen morphology, microsporogenesis and tapetal characters in Pandanales in a systematic context. Probable plesiomorphic character states include monosulcate apertures, perforate to reticulate sculpturing, a columellate exine with a foot layer, successive microsporogenesis, and a secretory tapetum. These correspond to generalised monocot or lilioid pollen and anther character states and all occur within Dioscoreales, the sister group to Pandanales. Within Pandanales, there are apparently homoplastic trends in each family towards either reduction in aperture size or covering the aperture with exine, resulting in a reduction in the delicate apertural area exposed to desiccation and entry of pathogens. Apomorphic character states in Pandanales include ulcerate, inaperturate or operculate pollen, pollen surfaces with protruberances, exine reduced to a foot layer, granular exine, absence of endexine lamellae, and possibly an invasive tapetum.

8.5.1. Comparative genomics of angiosperm MADS Box genes

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One of the central issues in evolutionary developmental biology is understanding the extent to which changes in developmental genetic pathways have resulted in new morphologies. We have focused on investigating the MADS box genes, which encode transcription factors controlling various aspects of floral development. We are examining the extent to which gene

duplication, regulatory diversification and differences in protein function have been important in modifying the roles of MADS box genes during angiosperm evolution. As a first step, we have developed strategies to rapidly identify all MADS box genes from a given species in order to carry out comprehensive phylogenetic and functional analyses. Using these data, we have initiated analyses of the roles of tomato MADS box genes in flower development. In addition, we have developed methods to carry out functional analyses in non-model lower eudicot species. These approaches are paving the way for a greater understanding of how these genes have diversified to specify different aspects of floral architecture.

8.5.2. Genetic basis of shape differences in *Antirrhinum* species

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Our project aims to identify the genes responsible for organ size and shape differences between *Antirrhinum* (snapdragon) species. Genetic natural variation was dissected by crossing a large organ species (*A. majus*) with a small one (*A. charidemi*) and generating an F2 population and recombinant inbred lines (RILs). A molecular map has been constructed for these populations. Traits, such as organ area or length, are easily measured but shape is more difficult to describe numerically. We have therefore adapted statistical shape modelling methods to describe leaf and flower shapes. Each independent shape variation in our population is described by a Principal Component (PC). Thus, each flower or leaf shape is characterized by figures (corresponding to the deviations to the mean). This allowed us to identify QTLs responsible for these complex shape transformations (ie the PCs). Furthermore, marker-assisted selection of Near Isogenic Lines (87.5% *A. majus*) allowed us to accurately dissect and characterize the effects of QTLs generating inter-specific shape variation.

8.5.3. Developmental and molecular genetics of traits affecting mating system: Self pollination, floral symmetry and protandry

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The role of floral symmetry and the relative developmental flexibility of male and female phases within a flower are discussed in the context of plant mating system evolution. The genus, *Collinsia* (*Plantaginaceae*), contains species that differ dramatically within an individual flower's lifespan in the timing of autonomous selfing. I present data on the variation within and among species in floral developmental traits in a phylogenetic context. Our data suggests that developmental flexibility in herkogamy and dichogamy has allowed species to move between more selfing and more outcrossing modes. These results challenge the idea of selfing as an evolutionary dead end and suggest a role for developmental lability in the evolution of plant mating systems. Second, we are conducting molecular genetic investigations into the role of floral symmetry genes in affecting protandry. Floral symmetry genes like CYCLOIDEA, first identified in snapdragon, *Antirrhinum majus*, are implicated in timing of anthesis. A general model for the role of CYCLOIDEA in mating system evolution will be presented.

8.5.4. 'Great and curious blunder in dame nature' (C. Darwin 1860): An evo-devo analysis of the origin and diversification of andromonoecy.

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Andromonoecy is a sexual system in which plants produce both hermaphroditic and staminate flowers. Comparative analyses of hermaphroditic and andromonoecious species of the genus *Solanum* have provided insight into the developmental transitions associated with the origin and diversification of this sexual system. Andromonoecy in *Solanum* arose as a phenotypically plastic response to the resource demands of developing fruit. Due to existing developmental gradients within inflorescences, this plastic response was specific to ovary function of distal flowers. In the common ancestor of the andromonoecious taxa, the developmental innovation of pre-anthesis termination of gynoecial

maturation in distal flowers resulted in the evolution of morphologically staminate flowers. This mechanism drew upon a gradient of developmental potential already present in the hermaphroditic ancestor. The production of morphologically staminate flowers was initially a plastic response to the presence of developing fruit. Subsequently, this same phenotype of staminate flower production in distal positions became a fixed aspect of the phenotype in more derived taxa.

8.5.5. Genetics of reproductive barriers in *Petunia*

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Petunia (Solanaceae) has two frequently syntopic species (*P. integrifolia* (*P.i.*) and *P. axillaris* (*P.a.*)) that do not naturally hybridise. Gene flow is hindered ecologically by floral pollination syndromes for bees (*P.i.*) or hawkmoths (*P.a.*) and genetically by unilateral or bilateral pollen-style incompatibilities.

Genome segments of bilaterally incompatible accessions of *P.i.* and *P.a.* were bred into a laboratory strain of *P. hybrida*. Phenotypic and QTL analyses revealed a complex genetic basis for most components of the pollination syndromes (morphology, nectar, fragrance), except petal colour which is under major gene control. In contrast, genetic barriers are under relatively simple control and can be broken by allelic exchanges at two or three loci expressed in pollen or style. From combined data in *Petunia* we propose that step-wise ecological differentiation for insect pollinators has reinforced a primary isolation event based on pollen-style incompatibilities. We seek further evidence by identifying molecular components of both gene flow barriers in nearly isogenic lines.

8.5.6. Evolutionary genetics of carpels: using California poppy (*Eschscholzia californica* Cham.) as a basal eudicot model system

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The evolutionary origin of the enormous variety of carpel morphologies among flowering plants, and its molecular basis, are not clear. Carpel development genes are being defined in *Arabidopsis*, a higher eudicot. To test if these genes play a conserved role in carpel development, we have chosen to examine them in California poppy, a new model basal eudicot species that can be manipulated transgenically. Flower development in the poppy has been described in detail to allow interpretation of defects in poppy development following transgenic approaches. Also, poppy has been successfully transfected with *Agrobacterium* and transgenic calli have been obtained expressing the GFP gene. We have been able to identify homologs of the *Arabidopsis* carpel development genes AGAMOUS, CRABS CLAW, and SPATULA in the poppy. Comparison of the role of these genes in California poppy, *Arabidopsis*, and rice will help reveal core genes that underlie carpel development in all angiosperms.

8.6.1. Vascular plant cyanobacterial symbioses: ancient associations for ancient environments

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The origin, evolution and past significance of vascular plant-cyanobacterial symbioses (*Gunnera*, cycads) remains an enigma. Most evidence suggests that they were a globally-significant component of many ecosystems in the past, possibly up to the Cretaceous period, but now show a much more restricted distribution. Based on the occurrence of fossil pollen and the current restriction of many species to wet or waterlogged environments I propose that the reason for their past success was because of their ability to fix nitrogen in anoxic/sulphidic environments where N availability was likely to be severely limited because of the reduced decomposition of organic material. Such environments were more common in the past due to significantly higher sea levels, with inland seas or seaways a common feature of many continental interiors, particularly in the Cretaceous. The subsequent decline of vascular plant cyanobacterial symbioses is associated with increasing aridity and reduced sea levels. Viewed in this way the cycads and *Gunnera* may represent relict 'gymnosperm' and angiosperm responses, respectively, to environmental conditions that are uncommon today.

8.6.2. Evolution of cyanobacterial symbioses

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Cyanobacteria occur in symbiosis with a wide range of eukaryotic partners. The putative selective advantage for the eukaryote is added metabolic capacities: photosynthesis, sometimes with nitrogen fixation, for non-photosynthetic partners, and nitrogen fixation where the partner is photosynthetic in its own right or through another symbiosis. Evolution of symbiosis requires a recognition system, and the bypassing of any anti-biophage mechanisms. Continuation of the symbiosis involves either horizontal or vertical transmission of the cyanobiont. Horizontal transmission could involve problems with partners finding each other, but permits choice of partner genotypes appropriate to the local environment. Vertical transmission overcomes the problem of finding a partner, but restricts partner choice. Vertical transmission occurs via asexual or sexual propagules; in the latter case it is typically through eggs in oogamous organisms, but in at least one case it involves sperm. Plastids arose from vertically transmitted, obligately symbiotic cyanobionts, with transfer of many genes from the cyanobiont to the host nucleus, or gene loss from symbiosis.

8.6.3. Cycad genomics: a platform to study the rise of the seed plants and their symbionts.

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The cycads are the oldest extant seed plants, which exhibit characteristics intermediate in evolution between seed-less plants, vs. more derived plants such as angiosperms. Among the plesiomorphic characters found in cycads are dichotomous branching, circinate vernation, flagellated gametes and ovules borne on leaf-like sporophylls. Apomorphic characters found in cycads include the production of seeds, pollen and coralloid roots. Coralloid roots in cycads have nodule like structures that house nitrogen fixing bacteria and are the oldest living plants which form root nodules. In order to tap into the rich genetic history of cycads, we have created an end sequence tag (EST) database made from tissues of developing cycads. To date, we have sequenced nearly 10,000 ESTs for comparative genomic studies. Current analysis is ongoing to study these genes in the formation of reproductive and vegetative structures will help understand the evolution of such important characters such as ovules as well as the development of coralloid roots to begin to understand the intimate relationship between plants and nitrogen-fixing, symbiotic bacteria.

8.6.4. How evolved is the *Azolla-Anabaena* symbiotic system?

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Azolla is a heterosporous aquatic fern with a fossil record dating back to the mid-Cretaceous. The leaves are overlapping, each with a floating and a submersed lobe. In the chlorophyllous dorsal lobe (floating) exists an ovoid cavity with a filamentous nitrogen-fixing cyanobacteria, usually referred to as *Anabaena azollae*, and several genera of bacteria. This leaf cavity behaves as both the physiological and dynamic interface unit of this symbiotic association where the main metabolic and energetic flows occur. In this sense, it can be considered as a natural microcosm.

Traditionally considered as a 'lower' vascular plant, *Azolla* exhibits symbiotic characteristics more evolved than the other vascular plant-cyanobacterial symbioses. This unique symbiosis is sustained throughout the fern's life cycle, where the cyanobacteria and bacteria are always present, in contrast to the more evolved vascular plant groups that associate with cyanobacteria. In this sense, we can consider this symbiotic association as a successful co-evolved system that also makes important contributions to the ecological, biofertilization and biotechnological fields.

8.6.5. New perspectives in plant-cyanobacterial symbioses: the putative roles of insect dispersal and diazotroph replacement

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Many aspects of the biology of plant-cyanobacterial symbioses remain virtually unstudied and two potentially rewarding examples will be discussed. Firstly, flying insects are frequently seen around *Gunnera* rhizomes and some of them may respond to visual and/or chemical signals produced by unusual stem glands. These insects could effectively transport compatible *Nostoc* symbionts from old, decomposing rhizomes, to young seedlings. Pollination biology abounds with analogous examples that improve evolutionary fitness through associations involving insect vectors. Secondly, only two monotypic liverworts, constituting a basal group among the hepatics, invariably associate with *Nostoc*. However, the slime papilla, that has an important functional role in *Nostoc*-liverwort symbioses, constitutes a common feature of all extant liverworts. It is possible that the slime papilla of many derived liverworts still functions in N-fixation, but now forms symbiotic associations with non-cyanobacterial diazotrophs.

8.7.1. Problems of transferring Tertiary plant record into potential vegetation maps

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Problems that hinder producing maps of potential Tertiary vegetation are in different palaeogeographic configuration of land & sea and relief that changed by orogeny, in variation in global climate, atmospheric circulation and the world ocean, and the extinct flora. To overcome these problems, palinspastic maps and well-determined and -dated spectra of leaf, fruit & seeds and spore & pollen records are relevant. Each plant organ is differently biased and expresses parts of vegetation. It is difficult to transfer the fossil spectra quantitatively into a community due to overproduction of fossil organs and taphonomic processes, although abundance of elements may indicate vegetation changes (NAP vs. AP). Contrary to previous models of vegetation units that were intuitively coined on a fossil plant assemblage, the newly developed system for the European Neogene stresses differences between zonal, azonal and extrazonal formations, impact of precipitation and uses objective definitions based on diversity percentages of components.

8.7.2. Climatic changes in the Miocene of Central Europe - the potential of using fossil angiosperm wood

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Fossil angiosperm wood is often neglected as a source of complementary information in palaeoclimate reconstruction. Nevertheless its potential to improve climatic data is great. Climate variables can be determined using the statistical model based on angiosperm wood characters without any systematic background. Insufficient number of wood types is however the weakest point of this type of palaeoenvironmental reconstruction method. On the other hand, the Nearest Living Relative (NLR) approach is very sensitive to the exact systematic attribution of the fossil wood and to the recognition of its closest living analogue. The difference between the two methods is demonstrated in the case of the Miocene wood floras of the Czech Republic and Germany focusing on the time interval of Early to early Middle Miocene. The results are also compared to the climatic data based on other palaeobotanical approaches.

8.7.3. Neogene ferns : often neglected but not forgotten

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Many specialists on Neogene floras focus their studies on angiosperm fossils because these are very powerful tools for reconstructing vegetation and climate using both near living relative and physiognomic approaches. Both angiosperms and conifers figure strongly in Neogene plant community reconstructions. In contrast, ferns (and other spore bearing plants) are rarely studied in comparable detail and often do not appear in reconstructions. In this presentation examples of Neogene ferns will be reviewed. Examples will be chosen to demonstrate

Neogene fern communities and fern associations and to consider to what extent ferns may contribute to palaeoclimate reconstruction. These examples will be drawn from both the macrofossil and palynological records. Attention will also be drawn to unresolved issues, such as the origin, and recognition in the fossil record, of epiphytic and climbing ferns.

8.7.4. The Late Miocene Karlovo Basin (Bulgaria, SE Europe): vegetation and climate reconstructions based on pollen analysis

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The sediments of Bulgarian Neogene basins contain important floristic information for understanding the evolution of the local and regional Neogene flora, vegetation patterns, palaeoecology, and palaeogeography of the Balkan Peninsula. The present report focuses on palynological studies of the freshwater deposits of Karlovo Graben situated in the Central Bulgaria, and provides data on Late Miocene-Pliocene vegetation and climate.

Based on floristic data the main plant communities were outlined. The dominant species in the zonal vegetation were floristic elements growing in temperate or warm-temperate climatic conditions, while thermophilous floristic elements are sporadically presented. The studied palaeoflora shows a stage in the long-term evolution of the Neogene flora on Balkan Peninsula, connected with the reduction of palaeotropical elements, dominance of arctotertiary taxa in the vegetation structure, and increasing distribution of grass vegetation.

8.7.5. Late Miocene to late pliocene floristic, vegetational and climatic changes in northern and central Italy as indicated by plant macrofossils

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In northern and central Italy macrofossil assemblages (mainly carpofloras) have been recently detected and studied in some time intervals which were not covered by macrofloral documentation up to now. In addition, the stratigraphic context of several historical localities (Senigallia, Gabbro, Montajone, Upper Valdarno, Sarzanello, Mongardino, etc.), which provided large leaf collections, has been re-examined on the basis of recent geological work. Consequently, the analysis of macrofloral data now permits to provide a less fragmentary reconstruction of the floristic, vegetational and climatic changes in the late Miocene to Late Pliocene time interval. Long gaps in the documentation still exist in the Early Pliocene (from 5.3 to 4.0 Ma), in the early Middle Pliocene (3.5 to 3.1 Ma), and in the Late Pliocene of northern Italy (about 2.5 to 2.0 Ma). Most of these gaps are filled up by palynological data published by other authors. The good Late Miocene and Pliocene stratigraphic record in Italy will certainly permit detection of new assemblages in those time intervals which are still poorly known.

8.7.6. Climate and vegetation derived from a Pliocene-aged leaf flora from SE Shanxi Province, North China

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The Zhanguan Formation (36° 58' 01" N, 112° 50' 55" E), located at the boundary of Yushe and Wuxiang Counties in SE Shanxi Province, North China, was deposited in an intermontane lake basin. CLAMP (Climate Leaf Analysis Multivariate Program) was used to determine the palaeoclimate quantitatively and yielded an MAT (mean annual temperature) of 9.67°C ± 1.17°C (2) a WMMT (warm month mean temperature) of 21.84°C ± 1.58°C and a CMMT (cold month mean temperature) of -1.47°C ± 1.88°C. CLAMP also suggested a RH (mean annual relative humidity) of 71.66% (±7.4). The precipitation during the growing season was estimated to have been 764.5mm ± 336mm. A moderately distinct wet and dry season was indicated with the precipitation during the wettest three months estimated as 476.7mm ± 140mm and the three driest months totaling 361.9mm ± 93mm. The climate of Zhanguan area during the Pliocene is similar to that of Yushe in SE Shanxi today.

8.7.7. The integrated plant record: the ultimate tool to reconstruct Neogene zonal vegetation in Europe

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Palaeovegetation maps are presented based on the records of the leaf, fruit and pollen floras. During the interval 17-14 (-12.5) m.a., subtropical broad-leafed evergreen forests (BLEF) and partly subtropical subhumid xerophytic forests (SXF) were spread in more (south)-western parts of Europe, while (warm-)temperate broad-leafed deciduous (BLDF) and partly mixed mesophytic forests (MMF) prevailed from the Carpathians eastwards. BLEF had a wide N/S distribution. During 12-8 m.a. BLDF and MMF were widespread in Central Europe, reaching as far as Spain, while the distribution of BLEF shrank to climatically favourable regions. SXF are documented from the Balkan Peninsula, and landscape opening is indicated in southern parts of the Russian Plain. During the interval 7-4 m.a. MMF are widespread. BLEF were once again restricted to refuge areas. SXF extended largely beyond today's 45° latitude. Xeric open woodland was developed from central Italy southwards; records of grassland are available from the Russian Plain.

8.8.1. Are plant chromosomes different?

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Comparative genome analyses at various levels provide detailed insight into the changes in chromosome structure, which have occurred during evolution. Here, we ask what it is about plant chromosomes that distinguishes them from animal chromosomes and hence makes them unique. To answer this question, we will compare various features of the genomes/chromosomes of higher plants and higher animals, including (1) the composition and prevalence of the repetitive fraction of DNA, (2) the structure and organisation of centromeres and telomeres, (3) the higher order chromatin structure, (4) the existence and extent of epigenetic imprints and (5) the structure and behaviour of mitotic and meiotic chromosomes. As specific examples, we will give a detailed description of the architecture of plant telomeres, outlining how this differs from their mammalian counterparts, and we will address the roles played by the proteins Mre11 and Est1 during the genome remodelling events of mitosis and meiosis. Finally, we will ask to what extent genomics can help us to understand plant genome dynamics such as polyploidy.

8.8.2. Differences in rDNA amplification during common bean (*Phaseolus vulgaris* L.) evolution and domestication

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Common bean (2n=22) is the world's most important grain legume for direct consumption, especially in Latin America and Africa. Although little is known regarding its genomic organization, the evolution and the domestication history of this species have been intensively studied. *P. vulgaris* originated in Ecuador/Peru and dispersed both northwards and southwards establishing the Mesoamerican and Andean gene pools. In each region, single or multiple domestication events took place. In five random cultivars analysed previously, a high degree of variation in the number and size of 45S rDNA loci was observed. In the present work, the 45S and 5S rRNA gene loci have been chromosomally assigned by fluorescent *in situ* hybridization in more than 25 wild and cultivated accessions from different gene pools and of different geographical origins. Three to four 45S rDNA loci were present both in wild and cultivated Mesoamerican genotypes. In contrast, in the Andean group, from three to seven loci were identified in wild genotypes, but cultivars had six to eight loci only. The correlation between genomic rDNA distribution and the evolution of the species will be discussed.

8.8.3. On the karyotype evolution of three grasses with an unusually low number of chromosomes

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The low basic chromosome number of two of the genera *Zingeria* and *Colpodium* is unusual considering that the basic number of chromosomes of the Poaceae family is seven. Analyses of the karyotypes of *Z. biebersteiniana* (Claus) P.Smirn. (2n=4, 2C=3.5pg), *Z. trichopoda* (Boiss.) P.Smirn. (2n=8, 2C=5.3) and *C. versicolor* (Stev.) Schmalh. (2n=4, 2C=2.4pg) revealed next dynamic evolution of the genomes: (1) Only four of the eight chromosomes of *Z. trichopoda* are strongly labelled after GISH with genomic DNA of *Z. biebersteiniana*. Therefore, *Z. trichopoda* is of amphidiploid origin and evolved from a hybrid involving *Z. biebersteiniana* and another species with four chromosomes. (2) The 45S rDNA loci of the *Z. biebersteiniana*'s subgenome of *Z. trichopoda* vanished after amphiploidisation. (3) Although the ITS1-5.8S-ITS2 sequences of *C. versicolor* and *Z. trichopoda* are highly similar, GISH of *Z. trichopoda* with labeled genomic DNA of *C. versicolor* did not result in a subgenome-specific labeling of *Z. trichopoda* chromosomes. Thus, the contemporary species *C. versicolor* is a closely related but not the direct ancestor of the allopolyploid species *Z. trichopoda*.

8.8.4. Karyotype evolution in *Nicotiana*

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The genus *Nicotiana* (Solanaceae) has 76 species in 13 sections and makes an excellent model system to study plant evolution and speciation. The base chromosome number is n=12, but the numbers can vary at both the diploid and tetraploid levels. The genus' centre of diversity is S. America, most species occur in South/North America, and one large section, *Suaveolentes*, is found in Australia (and one species in Africa). The Australian distribution of *Suaveolentes* arose as a consequence of recent long-range dispersal. We have embarked on a systematic analysis of karyotype evolution in the genus by combining molecular phylogenies derived from multiple gene sequences and karyotype/chromosome analysis using FISH and molecular biology. We report patterns of chromosome divergence between diploid and polyploid sections and in sections of different ages. We show relationships between rates of speciation and of karyotype divergence.

8.8.5. Karyotype evolution in *Arabidopsis thaliana* and its close relatives as revealed by comparative chromosome painting

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The chromosome number of *A. thaliana* (2n=10) deviates from that of most other members of the genus and of related taxa with a presumed ancestral chromosome number of n=8. Little is known about evolutionary sequence of events that contributed to the chromosome number reduction in *A. thaliana*. We addressed this question by comparative multicolour painting of pachytene chromosomes from related taxa with n=5-8 chromosomes using pools of chromosome-specific BAC contigs arranged according to the comparative genetic map between *A. thaliana* and *Capsella rubella* (n=8). Homeologous chromosomes/chromosome regions of species with 6 to 8 chromosome pairs (*Hornungia alpina* n=6, *Turritis glabra* n=6, *Neslia paniculata* n=7 and *A. lyrata* n=8) were identified. Considering the homeology pattern of *C. rubella* and *A. lyrata* as the ancestral karyotype, we reconstruct the sequence of events along which the chromosome number was reduced from n=8 to 5 in the context of genome reshuffling and genome size decrease.

8.8.6. Evolutionary dynamics of telomeric sequences in *Othocallis* (Hyacinthaceae).

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Lack of *Arabidopsis*-type T₃AG₃ telomere sequences has recently been reported for the majority of investigated taxa of the monocot order Asparagales. Extensive cytogenetic and molecular analyses of chromosome termini of four *Othocallis* species (*O. amoena*, *ingridae*, *mischtschenkoana*, *siberica*) revealed that they are formed by long stretches of vertebrate-type (T₂AG₃)_n repeats. Additionally, telomerase activity specifically synthesising vertebrate-type telomeric sequence was detected in *O. siberica*. *In situ* hybridization revealed also the presence of few additional interstitial, or terminally amplified telomeric signals in some chromosomes of *O. siberica* and *O. amoena*. These additional sites, often adjacent to or situated within heterochromatin, are highly polymorphic in *O. siberica*, and are apparently composed of large blocks of vertebrate- and *Arabidopsis*-type telomeric repeats. Thus, they may reflect a recent evolutionary switch from *Arabidopsis*- to vertebrate-type telomeric repeats in this plant group.

8.8.7. Genetically induced structural rearrangements of alien chromosomes in common wheat

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Chromosomes have been rearranged in evolution, spontaneously or by some driving forces, resulting in diverse karyotypes with different numbers and structures of chromosomes. A genetic system causing chromosome breaks has been found in wheat. When introduced into common wheat (*Triticum aestivum*, 2n=6x=42) from grass species (the genus *Aegilops*), certain *Aegilops* chromosomes called "gametocidal chromosomes" induce chromosome breaks in gametes lacking the gametocidal chromosome. They also cause breakage in alien chromosomes, such as rye and barley chromosomes, added to common wheat. Such chromosome breaks are sometimes non-lethal, and resultant deletions and translocations are stabilized in subsequent generations. In this paper the gametocidal chromosome and its action are briefly reviewed, and the evolutionary implications of the gametocidal chromosomes are discussed with some examples of chromosomal structural rearrangements induced by the gametocidal chromosome in rye and barley chromosome addition lines of common wheat.

8.9.1. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview

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Missouri Botanical Garden, St. Louis, MO, United States.

The presentation will cover various aspects of the family, including problems relating to classification, delimitation of taxa, and utilization of morphological and molecular characters in phylogenetic studies. It will also address problems of homoplasy, genome duplications, most challenging genera in evolutionary studies (e.g., *Boechera*, *Heliophila*), and character choice. The major monophyletic clades will be identified, and a new family-wide realignment will be proposed. A survey of the molecular coverage of the family will be presented, including numbers of species, genera, and markers in relation to geography. Numerous examples, covering the family worldwide, will be discussed, including problems relating to the basal clades (e.g., *Aethionema*). The final appeal is to conduct a phylogeny based on key "diploid" species, especially with small genome size, that represent major monophyletic clades in the family and conduct comprehensive, multi-locus comparative studies that involve several to many chloroplast, nuclear, and mitochondrial genes.

8.9.2. Molecules and migration: modern approaches to biogeography in cruciferous plants

M. Koch;

Heidelberg Institute of Plant Sciences, Heidelberg, Germany.

This contribution summarizes major achievements in phylogeography among cruciferous plant species. The most prominent work is discussed and the attempt is to cover many major lineages of cruciferous plants (e.g. from *Arabis*, *Cardamine*, *Lepidium*, *Clusia*, *Capsella*, *Arabidopsis*, *Cardaminopsis*, *Rorippa*, *Dentostemon*, *Draba*, *Diplotaxis*, *Thlaspi*, or *Pennellia* and others) as well as most geographic regions from world-wide temperate regions contributing to the enormous species diversity of this family. Several issues are addressed such as source areas of genetic diversity, refugial areas, vicariant patterns and colonization

routes. Some remarks will also focus on dating some of these processes and will summarize some divergence time estimates important for research among cruciferous plants. This talk is dedicated to the 65th birthday of Herbert Hurka and is in honour of his contributions to our better understanding of evolution in the Brassicaceae.

8.9.3. Comparative genomics and karyotype evolution in Brassicaceae

M. A. Lysak;

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Since the sequencing of a small crucifer *Arabidopsis thaliana* was completed in 2000, *Arabidopsis* sequence data and resources have greatly expedited comparative genomics across plant families and particularly within *Brassicaceae*. The present contribution aims to give a short overview on the progress of comparative genomics in *Brassicaceae* five years after publishing the *Arabidopsis* sequence. The following aspects of genome evolution in *Brassicaceae* will be discussed: (i) the role of polyploidy and genome size variation, (ii) comparative genetic mapping and genome colinearity, (iii) comparative chromosome painting and reconstruction of karyotype evolution, and (iv) anchoring genomic data within robust phylogenetic trees. It will be shown that despite the steady progress, evolutionary genomics within *Brassicaceae* is still just an emerging field. Future perspectives of comparative studies on crucifers with particular emphasis on phylogenomics will be outlined.

8.9.4. Hybridization, reticulation and polyploidy in Cardamine and other Brassicaceae genera

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²Charles University, Department of Botany, Prague, Czech Republic.

Many examples of past or recent interspecific hybridization and polyploidization from the Brassicaceae demonstrate the evolutionary importance of these processes within the family. Several studies addressing the origin of polyploids or taxa of hybridogenous origin, their evolutionary and biogeographic history have been recently published. Hybridization and subsequent polyploidization have been often shown to be associated with Pleistocene climatic changes, and also examples are known where glaciations significantly shaped genetic variation patterns and current geographic distribution of several polyploids of pre-glacial origin. Reticulation and introgression complicate reconstruction of phylogenetic relationships within the genera, and strongly biased patterns might appear in some cases. Conflicting signals from chloroplast and nuclear markers, on the other hand, can reveal past hybrid or allopolyploid speciation. The present review is focused on the results from recent investigations in the genus *Cardamine* and other Brassicaceae genera, and illustrates the above-given evolutionary phenomena.

8.9.5. Using Arabidopsis to explore evolution within the Brassicaceae and beyond

J. L. Bowman;

UC Davis, Davis, CA, United States.

For approximately 20 years *Arabidopsis* has been a model system to investigate developmental and physiological questions in plant biology, leading to the identification of genes and genetic systems involved in many processes. I will discuss some progress in extending ideas arising from knowledge of developmental genetic systems in *Arabidopsis* to other species of the Brassicaceae and phylogenetically more distant taxa throughout land plants. Conservation and diversification of gene function extending from known functions in *Arabidopsis* is generally successful as compared to candidate gene approaches to novel morphologies, even in more closely related taxa. Specific examples will include the role of allopolyploidy in the evolution of floral ground plans in *Lepidium*, the role of CRABS CLAW in the evolution of nectary position in Brassicaceae and eudicots, and role of Class III HD-Zip genes in the evolution of meristems in land plants.

8.9.6. Evolution of flower development in Brassicaceae: a molecular genetics view

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Flower and fruit development in Brassicaceae have much to offer for students interested in the relationship between the phylogeny of developmental control genes and the evolution of morphological and functional features. Traits currently under investigation range from the time to flowering affecting the plant habit via the structure of inflorescences and flowers and self-incompatibility mechanisms, to fruit shape and devices for seed dispersal. Besides being a cornucopia for studying microevolutionary events flower development in Brassicaceae may even provide model systems to investigate some especially enigmatic and controversial phenomena of macroevolution, such as saltational changes generating 'hopeful monsters'. We will provide instructive study examples for diverse evolutionary phenomena, with a focus on *Capsella bursa-pastoris*, one of the most successful weeds on a global scale, and the long-standing pet plant of Herbert Hurka.

8.9.7. Brassicaceae phylogenetics and the Hurka lab

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Major progress in evolutionary research of Brassicaceae is reviewed from the standpoint of comparative phylogenetics. Recurrent evolutionary syndromes in the family can be recognized and characterized by \pm correlated organismic, phylogenetic and eco-geographical parameters. Examples demonstrate such syndromes as (1) outbreeding perennials with dysploidy and a strongly divergent anagenetic pattern; (2) outbreeding perennials with allopolyploidy and an expansive reticulate cladogenetic pattern; (3) partially apomictic perennials of hybrid origin with \pm stabilized polyploidy (ortho- and anorthoploidy) + aneuploidy and an expansive mosaic cladogenetic pattern; (4) inbreeding annuals with di- and polyploidy, and an expansive ana- to cladogenetic pattern; (5) perennials with paleopolyploidy and a relic stasigenetic pattern. Considerable evidence for such a comparative approach is already available, and much of it is due to the excellent research efforts of Prof. H. Hurka and his former and present students and collaborators.

8.10.1. A comprehensive systematic appraisal of the Fagales with particular attention to the Juglandaceae

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An improved phylogenetic understanding of the order Fagales has been developed through various molecular data sets and recently described macrofossils. Advances have included data and methods drawn from the distinct fields of paleobotany, molecular systematics, and biogeography as applied to case studies in the families Betulaceae, Fagaceae, Juglandaceae and Nothofagaceae. A review of this progress will be presented, highlighting long-standing questions. Collaborative research on Juglandaceae will be used to address the integration of fossils into potentially robust estimates of phylogeny based exclusively on living taxa. Specific goals are to (1) place fossils and compare multiple methods; (2) explore the impact of missing data (fragmentary taxa) on the topology; (3) simulate the effects of missing data using our molecular data sets; and (4) compare the impact of single and multiple fossil constraints in estimating the age of clades.

8.10.2. Paleobotany and former range of the Central American relict family Ticoendraceae (Fagales)

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¹Florida Museum of Natural History, Gainesville, FL, United States,

²Ludwig Maximilians University, Munich, Germany.

The extant Central American genus *Ticodendron* (Ticoendraceae) shares gynoecial and pollen characters with the Betulaceae but has foliage more closely resembling that of Fagaceae. Sequence data from multiple genes have placed *Ticodendron* sister to Betulaceae and both then sister to Casuarinaceae. The fossil record of Ticoendraceae has important implications for the divergence time of Betulaceae and Ticoendraceae, but fossils may have been unidentified, or misidentified, due to lack of modern comparative material (extant *Ticodendron* was not discovered and named until the 1980s). We report leaves from the middle and late Eocene of Oregon, and anatomically preserved fruits from the Eocene of Oregon (Clarno Formation) and Southern England (London Clay) that closely

resemble *Ticodendron*. These European and North American Tertiary occurrences indicate that *Ticodendron* is a remnant of the Eocene boreotropical forest.

8.10.3. Fagalean woods--diagnostic characters and a critical review of the fossil record

E. A. Wheeler;

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Wood anatomy of all families of Fagales is summarized and compared. Characters considered are vessel grouping and arrangement, vessel-ray parenchyma pits, imperforate element type, ray composition, and axial parenchyma distribution. Predominantly apotracheal parenchyma occurs in all families; septate fibers occur only in Nothofagaceae. The minute alternate intervessel pits of *Betula* differ from all other Fagales. Casuarinaceae, Fagaceae, Myricaceae, and Ticoendraceae share predominantly solitary vessels. Vessel-ray parenchyma pits with markedly reduced borders and of irregular shape occur in the Quercoideae. Fossil woods with characteristics of the Fagaceae (Quercoideae) occur in the Turonian of the northern Hemisphere and are widespread in the Tertiary. Distinctive Betulaceae, Corylaceae, Juglandaceae, and Myricaceae woods occur in the Eocene. Nothofagaceae wood occurs in the Late Cretaceous and Early Tertiary of Antarctica.

8.10.4. Molecular systematics and biogeographical patterns in the birch family (Betulaceae): integrating fossils and molecules

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of Natural History, Chicago, IL, United States, ⁵Royal Botanic Gardens,

Kew, Richmond, United Kingdom.

The family Betulaceae comprises six extant genera (*Alnus*, *Betula*, *Corylus*, *Carpinus*, *Ostrya* and *Ostryopsis*) and about 150 species and is a dominant group in deciduous forests of the Northern Hemisphere. We review previous work on the systematics of Betulaceae and provide an overview of its fossil history. The well-documented fossils record of the family dates back to the Cretaceous and all extant genera, except the Asian endemic *Ostryopsis*, are represented in palaeoflora. In addition, many extinct genera attributed to the Betulaceae have been documented; some are even widely distributed such as *Palaeocarpinus*. We present a phylogenetic analysis of the family based on plastid (*trnL-F* region) and nuclear (ITS) sequence data. We reconstructed ancestral distributions onto the phylogenetic tree obtained with molecular data using dispersal-vicariance analysis (DIVA). The fossil record of the Betulaceae was used in combination with results from DIVA to infer patterns of dispersal, extinction and vicariance important in the phylogeographic diversification of this family in comparison with patterns inferred for other families of the Fagales.

8.10.5. Anatomically preserved fagalean fruits from the Eocene of western North America and their systematic implications

R. A. Stockey, R. A. Mindell, L. L. Elliott;

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Large numbers of fagalean fruits have been identified in calcareous nodules from the Eocene Appian Way locality of Vancouver Island. This study using anatomy and external morphology has revealed the presence of at least three types of Fagaceae fruits. These include triangular *Fagus*-like fruits with three locules and six ovules; small, two-loculed, cupulate, spiny fruits; and indehiscent, single-seeded, cupulate fruits with branched spines similar to *Castanopsis*. One of the two juglandaceous fruits is a dorsiventrally flattened nutlet with characters intermediate between subfamilies Engelhardioideae and Juglandoideae (Tribe Platycareae). Three dimensional reconstructions of these fruits using cellulose acetate peels and AMIRA 3.1 visualization software have allowed comparisons with fossil fruits from other well-known Tertiary localities and those of extant taxa. Internal anatomy and three-dimensional reconstruction make these among the best known fossil fagalean fruits, and their unique character combinations provide important phylogenetic information toward our understanding of the evolution of Fagales.

8.10.6. The fossil record and systematics of Fagaceae**Z. Zhou;**

Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, China.

The Fagaceae family has a rich fossil records throughout the Tertiary of the Northern Hemisphere. A critical review of the fossil records provides a basis of understanding the evolutionary and biogeography of the beech family. Cupulate fruits and flowers of two extinct genera, *Protofagacea* and *Antiquacupula* from late Santonian stage of the, late Cretaceous of Georgia are the oldest unequivocal remains of Fagaceae. They are different in several significant characters and indicate that the divergence of at least two lineages of Fagaceae have been established by Late Cretaceous. It is obviously that an origin of Fagaceae no later than the Late Cretaceous. Fossil records and modern distributions of each genus of the family were discussed. The family achieved its greatest generic diversity in Eastern and Southeastern Asia and all modern genera had broader distributions in the Tertiary than today. The paleogeographical distribution of Fagaceae indicates that the family originated in the Northern Hemisphere.

8.10.7. Evolutionary history of the Casuarinaceae**R. Paull, R. S. Hill;**

The University of Adelaide, Adelaide, Australia.

The Casuarinaceae are a morphologically distinct family with a long fossil record. Late Cretaceous pollen from Australia is indistinguishable from Betulaceae, the proposed sister taxon. The oldest Casuarinaceae macrofossils (Late Palaeocene, SE Australia) are assigned to the extant genus *Gymnostoma*, but fall outside its extant morphological range. *Gymnostoma* macrofossils also occur in South America and New Zealand (plus others with less certain affinities in the latter). *Gymnostoma* probably evolved in response to low soil nutrients, especially low P and N. Extant species occur on SW Pacific islands, New Guinea, New Caledonia, Malaysia and NE Australia. The oldest records (probably Oligocene) of two other genera, *Casuarina*, *Allocasuarina*, are from NE/SE Australia. Fossil and molecular data indicate they evolved tens of millions of years later than *Gymnostoma*, in response to low water availability and increasing aridity. Both genera are now widespread in Australia. *Casuarina* is also found in SE Asia and Pacific islands. The history of the fourth enigmatic genus *Ceuthostoma*, with two extant species in New Guinea and Indonesia, remains uncertain.

8.11.1. Apomixis in higher plants**Y. Savidan;**

AGROPOLIS-IRD, Montpellier, France.

Apomixis is commonly defined as asexual reproduction through seeds. Seed embryos result from the parthenogenetic development of unreduced egg cells in the embryo sacs. While both the failure of meiotic reduction and the failure of fertilization are needed, additional factors seem to be required for a successful apomixis expression. Save rare exceptions, apomicts are polyploids, and we don't really know why. Most of them exhibit a facultative apomixis, as part of their progeny is coming from a normal sexual development. Over 500 species have been reported to reproduce apomictically, in more than 40 different families, but high frequency of apomixis can only be found in three or four of these families. When apomixis and true sexuality are found together in the same species or agamic complex, Mendelian genetics usually suggests a single gene control, but molecular genetics shows that this control is located on a non recombinant segment of chromosome. While several molecular studies are underway aiming at transforming apomixis into a tool for breeding and crop propagation, too many questions that relate to the biology and expression of apomixis remain unanswered.

8.11.2. Perspectives on apomixis and taxonomy from Rosaceae subfamily Maloideae: better tools mean a less thorny problem**N. Talent^{1,2}, T. A. Dickinson^{2,1};**¹University of Toronto, Toronto, ON, Canada, ²Royal Ontario Museum, Toronto, ON, Canada.

In spite of the handicap of long generation times, the knowledge base about certain genera of Rosaceae subfamily Maloideae (*Malus*, *Pyrus*) is considerable because of their great economic importance. The subfamily, as recently revised, is a natural group

comprising several small genera and a few large ones (e.g. *Amelanchier*, *Cotoneaster*, *Crataegus*, and *Sorbus sensu lato*). While the former appear to be sexual diploids, the latter are taxonomically complex since they include both sexual diploids and apomictic polyploids. New tools that enable us to survey molecular and cytological variation are giving us a better understanding of them. In *Crataegus* we have been able to use flow cytometry to augment embryological studies with the ploidy levels of embryos and endosperm in mature seeds. This gives us a view of reproduction that is closer to its final outcome. We hope to shed light on the origins of polyploidy, the reproductive barriers, if any, between ploidy levels, and on the possibility of different solutions to the problem of endosperm balance with apomixis.

8.11.3. Unique reproductive system in dogroses (Rosa sect. Caninae) maintains successful and highly heterozygous genotypes**H. E. Nybom;**

Balsgard-Dept Crop Science, Kristianstad, Sweden.

We have studied the mostly pentaploid ($x = 7$, $2n = 35$) dogroses, *Rosa* section *Caninae*, where apomixis is only one of the means to maintain heterozygosity in homogeneous offspring. In four out of five investigated (micro)species, about 10 % completely maternal (according to RAPD and microsatellite DNA loci) offspring were encountered in interspecific crosses. The sexually derived offspring also resemble the seed parent more than the pollen parent. Due to the unique meiosis, three sets of 7 chromosomes occur as univalents and form 'passenger genomes' which are inherited only from the seed parent. The other 14 chromosomes form bivalents and constitute a diploid, highly homozygous genome, which takes part in recombination. Taxonomic distances are closely reflected by level of allele-sharing between univalent-forming chromosomes. Bivalent-forming chromosomes instead vary much more within species and less between. Segments of bivalent-forming chromosomes are likely to be exchanged within and even between taxa like in other outcrossing plant species groups. The univalent-forming chromosomes are instead passed on maternally, as in apomictic species.

8.11.4. Divergence and reticulation in the Erigeron sect. Phalacroloma apomictic complex.**R. D. Noyes;**

University of Colorado, Boulder, CO, United States.

Reticulation has long been hypothesized to be important in the origin and diversification of apomictic complexes. *Erigeron* sect. *Phalacroloma* (Asteraceae) includes sexual diploid and apomictic triploid and tetraploid plants centered in the southeastern United States. This group traditionally comprises *E. annuus*, *E. strigosus*, and *E. tenuis*. Phylogenies for sexual diploids based on rDNA spacer sequences (ITS and ETS) reveal five distinct rDNA haplotypes that likely diversified from 650 to 925 mya. Sexual diploids that possess combinations of haplotypes, furthermore, are consistent with widespread reticulation at the sexual diploid level. Apomictic plants are diverse, exhibiting single or multiple rDNA haplotypes corresponding to those found in diploid plants, as well as novel haplotypes of unknown origin. Although the complex phylogenetic patterns are consistent with multiple origins of apomixis, they could also be explained by the widespread introgression of apomixis genes that have a single common origin. These alternative hypotheses are being tested in the group using comparative genetic mapping.

8.11.5. Evolution of polyploid agamic complexes using Antennaria rosea (Asteraceae) as a model group.**R. J. Bayer¹, G. T. Chandler²;**¹CSIRO, Plant Industry, Canberra, ACT, Australia, ²University of North Carolina at Wilmington, Wilmington, NC, United States.

Antennaria (Asteraceae: Gnaphalieae) is useful as a tool for studying polyploid agamic complexes because both polyploidy and agamospermy are widespread in this genus. The diploids are morphologically well-defined and tend to be geographically restricted. The five polyploid complexes in the genus vary considerably with respect to ploidy levels, morphological variation and reproductive modes. The triploid/tetraploid gametophytic apomict, *Antennaria rosea*, is one of the most thoroughly examined complexes in the genus. The *Antennaria rosea* polyploid complex has a wider geographic range and possibly greater ecological amplitude than its diploid progenitors and is the result of

multiple hybrid origins from as many as eight sexual diploid species. The diploids gave rise to *Antennaria rosea* through the processes of interspecific hybridization and backcrossing, and also through hybridization with facultatively sexual apomicts.

8.11.6. Evolutionary aspects in *Hieracium* subgenus *Pilosella*

J. Fehrer¹, B. Gemeinholzer², K. Krak^{1,3}, S. Bräutigam⁴;
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The hawkweed subgenus *Pilosella* (Lactuceae, Asteraceae) is known for its notoriously complicated taxonomic structure due to ongoing reticulate evolution combined with a facultatively apomictic mode of reproduction. Recently, molecular approaches at clone, population and species level have begun to shed some light onto these processes. Gene flow across ploidy levels is common, and parental species of hybridogenous taxa often include apomicts, even as seed parents. Sexual taxa (diploid or polyploid) usually show high genetic variability. Apomicts vary from near clonality across large geographic distances to multiple origins on a small scale. *Pilosella* chloroplast haplotypes form two major groups whose separation predated most of the subgenus' speciation. Comparison of chloroplast and nuclear markers suggests two ancient hybridization events predating most of the speciation observed in the subtribe Hieraciinae: one between the *Hieracium*/*Chionoracium* subgenera ancestor and partly differentiated *Pilosella*, and a subsequent one between this introgressed *Pilosella* lineage and the closely related *Andryala* genus ancestor.

8.11.7. Identifying genes associated with apomixis in *Hieracium*

R. Bicknell¹, A. Koltunow², A. Catanach¹, S. Erasmuson¹, P. Fletcher¹, S. Johnson²;
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Hieracium is a daisy genus endemic to Eurasia and North America. The plants are small, easily cultivated and amenable to a wide range of experimental procedures. Seed formation within the genus *Hieracium* subgenus *Pilosella* occurs primarily by facultative apomixis, specifically by the mechanism of apospory (of the *Hieracium* type) coupled to autonomous endospermy. We study these plants as a model system for apomixis. The talk will present our understanding of the expression of genes within the *Hieracium* ovule during the initiation and progression of apomixis, the inheritance of apomixis determinants and the mapping of these regions. Particular emphasis will be given to our recent findings on the interactions occurring between the expression of sexuality and apomixis, and on inter-relationships between the components of apomixis in this system. The implications these data have for the possible engineering of this trait into target crops will also be discussed.

8.12.1. Nutrient limitation and stoichiometry of carnivorous plants: is it time to reassess the cost-benefit model for their evolution?

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The cost-benefit model for the evolution of carnivorous plants posits a trade-off between photosynthetic costs associated with carnivorous structures and photosynthetic benefits accrued through additional nutrient acquisition. An implicit assumption of this model is that nitrogen is the primary limiting nutrient. A review of published data and results of on-going research show that phosphorus, potassium, or calcium often is the primary nutrient limiting carnivorous plant growth. Stoichiometric analysis further suggests that more than one of these nutrients may co-limit growth, depending on local and changeable environmental conditions. Further, variability in the production of carnivorous structures has been demonstrated in *Sarracenia*, *Utricularia*, *Drosera*, and *Pinguicula*, and this plasticity can be mediated either by the absolute or relative concentrations of limiting nutrients. These data are used to develop a model for the evolution of botanical carnivory that reflects stoichiometric relationships and evolutionary compromises in addition to trade-offs in nitrogen uptake and photosynthetic efficiency.

8.12.2. A Study on the pollination biology of selected *Utricularia* species (Lentibulariaceae) on Lateritic Plateaus in the Western Ghats, India

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¹Rostock University, Institute for Biodiversity Research, Rostock, Germany, ²Agharkar Research Institute, Puna, India, ³Bonn University, Nees-Institute, Bonn, Germany.

The pollination biology of carnivorous plants is, to a large extent, an unexplored field. An exemplary study on the pollination biology of three gregariously flowering terrestrial *Utricularia* species was conducted in the Indian Western Ghats. Flower visitors were observed and identified. In order to test for self-pollination exclusion experiments were conducted. In addition, data on anthesis, phenology, flower volatiles and nectar quantity and its sugar content were obtained. Nectar quantities were extremely small (< 1 µl), sugar content varied from 8% to 74%. Self-pollination was excluded by spatial arrangement of reproductive organs, but can be mediated by visiting insects since all species are self-compatible. The flowers are visited by a broad spectrum of insects, several species of bees, butterflies, moths and hawk moths could be proven to be pollinators, though of different efficiency. This contradicted the morphology-based hypothesis of a highly specialized pollination syndrome.

8.12.3. Phylogeny, biogeography and character evolution in the carnivorous genus *Pinguicula* L. (Lentibulariaceae)

L. Legendre¹, K. Müller², T. Borsch², W. Barthlott²;
¹Laboratory of Plant Biology of aromatic and medicinal herbs - EA3061, Saint Etienne Cedex 2, France, ²Nees-Institute for Biodiversity of Plants, Universität Bonn, Meckenheimer Allee 170, 53115 Bonn, Germany.

The Butterworts (*Pinguicula*) constitute one of the three genera of the carnivorous Lentibulariaceae, comprising approximately 80 species. Phylogeny inference using nucleotide sequences of the chloroplast gene *matK* and the *trnK* group II intron revealed six well supported major lineages within the genus. These lineages largely reflect radiations in clearly defined geographic regions (Mexican/Caribbean, Eurasian, East Asian, European, SE USA and the south American Andes, Mediterranean and NE Atlantic coast), whereas most previously recognized sections of the genus are para- or polyphyletic. A set of 32 morphological characters were defined and their evolution within the genus evaluated in the light of the phylogenetic data. It is suggested that the genus *Pinguicula* may have originated from an ancient paratropical vegetation belt and later migrated southward along the Andes. Major diversification events appear to relate to the growth habit with an adaptation to freezing (winter resting bud) or drier environments (succulent and non-carnivorous leaves).

8.12.4. Recent progress in understanding the evolution of Lentibulariaceae (Lamiales)

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Carnivorous plants have emerged as model systems for addressing many ecological and evolutionary questions, and since Lentibulariaceae comprise more than half of all known carnivorous species, they are of particular interest. Studies using various molecular markers have established that Lentibulariaceae and their three genera are monophyletic with *Pinguicula* being sister to a *Genlisea-Utricularia*-clade, while the closest relatives of the family remain uncertain. Character states of the carnivorous syndrome in related proto-carnivorous lamialean families apparently emerged independently. *Genlisea* and *Utricularia* exhibit substitutional rates that rank among the highest in angiosperms for the molecular markers analyzed. In *Utricularia*, the terrestrial habit has been reconstructed as plesiomorphic, and an extension of subgenus *Polypompholyx* is warranted. In the protozoan-attracting *Genlisea*, subgenus *Tayloria* is revealed as basal lineage. In *Pinguicula*, the six major lineages found reflect radiations in clearly defined geographic regions, whereas most previously recognized subgeneric taxa are non-monophyletic.

8.12.5. Adaptive evolution of cytochrome *c* oxidase: Infrastructure for a carnivorous plant radiation

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The highly conserved respiratory machinery of eukaryotic cells might seem an unlikely target for selection supporting novel morphologies. We demonstrate that a dramatic molecular evolutionary rate increase in subunit I of cytochrome *c* oxidase (COX) from an active-trapping lineage of carnivorous plants is caused by positive Darwinian selection. Bladderworts (*Utricularia*) trap plankton when water-immersed, negatively pressured suction bladders are triggered. The resetting of traps involves active ion transport, requiring considerable energy expenditure. As judged from the quaternary structure of bovine COX, the most profound adaptive substitutions are two contiguous cysteines absent in ~99.9% of databased COX I sequences. This motif lies directly at the docking point of COX I helix 3 and cytochrome *c*, and modeling of bovine COX I suggests the possibility of an unprecedented helix-terminating disulfide bridge that could alter COX/cytochrome *c* dissociation kinetics. Thus, the key adaptation in *Utricularia* likely lies in molecular energetic changes that buttressed the mechanisms responsible for the bladderworts' radical morphological evolution.

8.12.6. Genomes of Lentibulariaceae: some smaller than in *Arabidopsis thaliana*, and with chromosomes of bacterial size

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Angiosperms are currently estimated to vary about 800-fold in nuclear holoploid genome size (C-values), with the smallest established 1C-value of 155 Mbp recorded in *A. thaliana*. In Lentibulariaceae now three taxa were found ranging significantly lower: *Genlisea aurea* with 65 Mbp, *G. margaretae* with 67 Mbp, and *Utricularia gibba* with 88 Mbp. The smallest anaphase chromatids in *G. aurea* have 2.1 Mbp and are thus of bacterial size (NB: *E. coli* has ca 4 Mbp). Several *Utricularia* spp. range somewhat lower than *A. thaliana* or are similar. The highest 1C-value, in *G. hispidula* with 1506 Mbp, results in 23-fold variation for *Genlisea* and the Lentibulariaceae. Genome size variation in angiosperms is now more than 1900-fold. It is relevant for the understanding of genome miniaturization, that the *Genlisea-Utricularia*-clade exhibits among angiosperms in several genes the highest mutational rates, while this is not the case in *Pinguicula*, which varied in C-values only 1.4-fold from 487 to 693 Mbp.

8.12.7. Molecular phylogeny and structural evolution of carnivorous plant families within the Caryophyllidae s.l.

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Using comparative sequencing of the *matK* gene, hypotheses of relationships between the carnivorous Droseraceae, Nepenthaceae and Dioncophyllaceae and ten other families of the Caryophyllidae s.l. were tested. Parsimony analyses gives evidence that the syndrome of carnivory has a monophyletic origin. Tree topology indicates Droseraceae in a most basal position and Nepenthaceae as sister to Ancistrocladaceae/Dioncophyllaceae. The exclusion of *Drosophyllum* from Droseraceae suggests no close relationship with this family. Concerning Dioncophyllaceae and Ancistrocladaceae, carnivory was lost in the evolutionary process, with the exception of *Triphyophyllum*. The close relationship of these taxa is also corroborated by several morphological and phytochemical characters. An evolutionary process of carnivory starting from adhesive glands via glands with digestive properties to special trapping devices is outlined.

8.13.1. Photoprotective energy dissipation and the bigger picture

B. Demmig-Adams, V. Ebbert, K. E. Mueh, W. W. Adams III; University of Colorado, Boulder, CO, United States.

The process of photoprotective thermal energy dissipation will be placed in the context of the network of reactions serving in photoprotection and signaling. Several different types of thermal dissipation, including flexible and sustained processes, and their occurrence in different environments and different groups of plant species will be presented. The involvement of xanthophylls, trans-thylakoid pH gradient, and the PsbS protein as well as a possible role of other proteins will be discussed. Other proteins considered include (a) members of the PsbS-related subgroup of stress-induced proteins of the light-harvesting protein family and (b) proteins of the photosystem II core and their phosphorylation state. The association of sustained thermal dissipation in evergreen species with the photoinhibition of photosynthesis will be addressed, and the relationship between these processes and events at the level of the cell, leaf, and whole plant discussed. Parameters considered include leaf carbohydrate levels, leaf vein density, and whole plant source/sink balance.

8.13.2. Redox controls set the threshold for cell expansion, programmed cell death and systemic resistance to pathogens in *Arabidopsis thaliana*

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Programmed cell death (PCD), senescence and reactive oxygen species (ROS) are linked through complex genetic controls. Plants have optimized strategies for redox regulation, including mechanisms for controlling the generation of and a network of pathways of ROS detoxification. Vitamin C (L-ascorbic acid, AA) is the major redox buffer of the plant cell modulating physiological and stress responses in the cytoplasmic and apoplastic compartments. The *Arabidopsis* mutants deficient in AA (*vtc* *vtc1* and *vtc2*, contain about 30% of wild type AA levels and exhibit micro lesions, expression of PR proteins and enhanced basal resistance against infections caused by *Pseudomonas syringae*. Evidence will be provided that cell death in the *vtc* mutants is not linked to senescence but rather to low redox buffering capacity. Low ascorbate limits cell expansion and triggers programmed cell death in a co-ordinated manner activating systemic enhanced resistance and enhancing basal resistance when cell growth has ceased.

8.13.3. Oxidative stress networks and nuclear control

B. J. Pogson;

The Australian National University, Canberra, ACT, Australia.

Limiting damage caused by oxidative and abiotic stress requires a complex set of physiological, biochemical and transcriptional responses. We are investigating components of the high light stress response and the extent to which drought and high light induce unique or common pathways. A combination of forward and reverse genetics in *Arabidopsis* and *Chlamydomonas* has identified mutants and genes that alter responses to a range of oxidative stresses. Ascorbate peroxidase 2 (APX2) is induced by excess light and catalyses the conversion of hydrogen peroxide to water. Thirteen *alx* (altered APX expression) mutations were identified by luciferase imaging. A gain-of-function mutation, *alx8*, has a five fold increase in APX2 expression under high light and is also extremely drought tolerant. With respect to loss-of-function, mutations and RNAi lines of high light-induced genes have one or more of the following: reduced APX2 expression, increased photobleaching, or reduced capacity to accumulate anthocyanins under stress. The suites of unique and common phenotypes demonstrate abiotic stress pathways are not simply linear pathways but are multifaceted networks.

8.13.4. Control of photosynthetic gene expression by the environment

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No abstract received.

8.13.5. Plant peroxiredoxins in plant metabolism and stress tolerance

K. J. Dietz, I. Finkemeier, M. Laxa, P. Lamkemeyer, S. Jakob, M. Baier;
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Redox signalling and antioxidant defence are intricately involved in many aspects of plant metabolism particularly under stress such as salinity, heavy metals and excess light. The new group of peroxiredoxins (Prx) as thiol-based peroxidases play an important role in maintaining the redox homeostasis and possibly as redox sensors in signalling. Four examples illustrate indispensable Prx function in *Arabidopsis thaliana*. The mitochondrial Prx IIF is essential for normal root growth under stress. The cytosolic Prx IIC modifies plant response to salinity. The chloroplast 2-Cys Prx and Prx Q are related to photosynthesis and act as redox sensors modulating photosynthetic activities. Apparently, Prx have adopted diverse compartment-specific metabolic and signalling functions that cannot be substituted for by any other antioxidant present in the plant cell.

8.13.6. Plant phenylpropanoid metabolism in relation to oxidative stress

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Phenylpropanoids and flavonoids play important structural roles in plants, as well as in defense against a variety of biotic and abiotic stresses. A wide range of environmental factors that predispose plants to oxidative stress, i.e., high light, UV radiation, low temperatures, ozone, and pathogens, have been shown to induce the synthesis phenolic metabolites with antioxidant properties. Until recently, phenolic compounds were not considered part of the antioxidant network of plants. However, biochemical studies of gene expression and physiological studies of plant stress responses suggest that certain phenolics may serve a primary role as antioxidants in plants. It is likely that phenolics act in concert with other protective molecules in plant cells, including enzymatic and non-enzymatic scavengers of reactive oxygen species, perhaps compensating for deficiencies of such molecules during periods of stress. A future challenge will be to elucidate mechanisms by which the antioxidant activity of phenolic compounds is coordinated in cellular environments and to identify how oxidative signals regulate the expression of phenylpropanoid defense genes.

8.13.7. Altered light acclimation in transgenic potato (*Solanum tuberosum* L. var. *Desirée*) plants with decreased leaf ferredoxin (Fd) content

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Pflanzenphysiologie, Fachbereich Biologie/Chemie, Universität Osnabrück, Germany.

The effect of high light was analyzed in antisense potato plants with reduced leaf Fd contents. After a transfer of low-light acclimated plants into high light, the mutants developed an elevated PSII-redox pressure, but the stroma remained more oxidized. The amount of PSI proteins increased slightly, while the LHC-II level decreased. The chloroplast and nuclear-encoded thylakoid-protein levels changed in the same way in wildtype (WT) plants and in the mutants, indicating that chloroplast and nuclear gene expression are still coordinated. The major differences were an increased binding of Fd-NADP⁺ oxidoreductase to the Cyt b/f complex and a higher level of PSII phosphorylation. No indication for ROS formation was found. After pronounced high-light treatment, the total amount of thylakoids decreased, but their composition was still similar to WT plants. All changes were fully reversible within one week. The lack of Fd in the antisense plants induces a pronounced light acclimation, although the electron pressure only of intersystem carriers, but not of the stroma, was increased, and no ROS accumulated. A novel type of PSI modification may prevent ROS formation.

8.14.1. Interactions between global environmental change and biodiversity.

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Université Paris-Sud XI, Orsay, France.

The purpose of this talk will be twofold: 1) to examine the effects of global environmental change on biodiversity and 2) suggest ways in which changes in biodiversity could alter the future trajectory of

atmospheric and climatic change. Experiments, models and observations all suggest that global environmental change has and will continue to strongly modify biodiversity in terrestrial ecosystems. This has raised considerable concern about the conservation of species and ecosystems in a rapidly changing atmosphere and climate. I will provide an overview of current views on the effects of global environmental change on biodiversity and highlight key areas where our understanding is weak. It is now becoming increasingly clear that these changes in biodiversity - as well as those due to introduced species, land use change, etc. - will modify the functioning of ecosystems. I will review evidence that changes in biodiversity alter ecosystem functioning and response to perturbations, focusing on how biodiversity loss might alter plant productivity and ecosystem carbon storage responses to global change.

8.14.2. Global change and genetic erosion of dominant and keystone species

E. Huber-Sannwald, J. T. Arredondo Moreno, A. G. Alpuche Solis;
IPICYT, San Luis Potosi, S.L.P., Mexico.

Biological diversity at the landscape, community, species and genotype level has high priority in global change research as human induced changes in biodiversity may jeopardize the stability of ecosystems. A vast literature supports the notion that loss of plant species diversity negatively affects primary production. However, the relationship of genetic diversity and ecosystem function is poorly understood. Clear differences in tissue chemistry among genotypes from different grazing systems suggest that the genetic composition of populations may influence biogeochemical cycles. Hence, a decline in genetic diversity may adversely affect ecosystem processes especially in naturally species-poor communities such as semiarid grasslands in the Americas. With a cross-disciplinary approach spanning from molecular to ecosystem ecology we demonstrate that overgrazing may lead to genetic erosion of keystone species in grasslands with clear impacts on ecosystem processes. We discuss the emerging role of genotype diversity in the context of ecosystem function and for the maintenance of ecosystem services in a globally changing environment.

8.14.3. Invasive plant species, global change and biodiversity

R. J. Hobbs¹, H. A. Mooney², E. S. Zavaleta³;
¹Murdoch University, Murdoch, Australia, ²Stanford University, Stanford, CA, United States, ³University of California, Santa Cruz, CA, United States.

Invasive plant species are an important cause of biodiversity change at local, regional, and global scales today. A range of factors could inhibit or promote invasions at the introduction, establishment and spread stages in the next 100 years. Many trends contribute to the increase in introductions of potentially invasive alien species, particularly increases in global trade and the continuing introduction of plant species as new crop, pasture or horticulture varieties. The increased movement of organisms across former biogeographic barriers is matched with land use and global change trends that favor establishment and spread of newly introduced species. The future extent and impact of invasive alien species will depend on whether these exacerbating factors can be offset by effective new management practices and improved local and global institutional responses. Future biological diversity at all scales will depend on what paths we choose.

8.14.4. Impacts of anthropogenic nitrogen deposition on invasive and native species in southern California desert and shrublands

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University of California, Riverside, Riverside, CA, United States.

Anthropogenic nitrogen deposition occurs at levels up to 30 kg ha⁻¹yr⁻¹ in the Mediterranean-type shrublands and Mojave Desert vegetation of southern California, mostly as dry deposition during summer. The native vegetation, which includes some 200 rare plant species, has been gradually declining during the last 40 years downwind of urban areas, with a concomitant increase in Mediterranean annual grasses (e.g., species of *Schismus*, *Bromus*) and forbs (*Erodium*, *Brassica*). To test the relationship between soil N and vegetation change, surveys were done along N deposition gradients in desert and shrubland. Extractable soil N (nitrate plus ammonium) ranged from 1 to 19 mg/kg in desert and 10 to 39 mg/kg in shrubland. Native plants declined from 92 to 34

species per site in shrubland, with a concomitant increase in exotic grass cover from 1 to 69%; diversity losses were similar in the desert gradient. The mechanisms of vegetation change include increased competition between nitrophilous exotic species and native species, and increased fire frequency of the productive N-fertilized grasses that prohibits future recruitment of native species.

8.14.5. Patterns of African plant diversity and the impact of future climate change - problems and perspectives

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Where are the centers of plant diversity in sub-Saharan Africa and how will these patterns be affected by future climate change? We mapped large-scale diversity patterns of sub-Saharan Africa based on distribution data of more than 4500 plant species (c.10% of the flora). Bioclimatic range models were generated for each of the species to control for effects of disparate regional collection intensity. Further, species distributions were modeled under future climate scenarios. Centers of species richness are currently located in parts of the Guineo-Congolian rain forests, east African afro-montane areas, and in Southern Africa. Many of these centers are already heavily threatened due to habitat conversion. Future climate scenarios, in addition, suggest a decline in climate suitability for many species in recent centers of diversity in lowlands, resulting in decreasing species richness. In contrast, afro-montane centers of diversity are less affected. Sustainable conservation concepts should comprise land-use-induced and climate-change induced aspects of habitat conversion. Afro-montane areas may be key areas in this context.

8.14.6. Spatial and temporal variation in the productivity-diversity relationship in Mediterranean plant communities

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¹Hebrew University of Jerusalem, Rehovot, Israel, ²Tel Aviv University, Tel Aviv, Israel.

Climate models predict changes in rainfall patterns of arid and semi-arid regions in the Mediterranean basin that should lead to changes in primary productivity and diversity of their plant communities. Although rainfall is one of the main drivers of ecosystem functioning in these regions, there is yet no agreement as to how the productivity-diversity relationship is affected by rainfall. We propose that analysis of the variation in the relationships between rainfall, productivity and diversity occurring among years (temporal scale), across a steep geographic rainfall gradient (spatial scale) and after local rainfall manipulations should allow predictions about possible patterns of change in plant community structure and productivity due to climate change. Current results suggest that: a) the productivity-diversity relationship of the herbaceous plant community varied with rainfall regime across the gradient, but was less or not affected by inter-annual variation and rainfall manipulations, and b) variation in productivity was associated with changes in the frequency of species-functional traits in the community.

8.14.7. Coastal dynamics of the inland lakes in Central Asia

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 University of Bielefeld, Department of Ecology, Bielefeld, Germany.

The inland lakes of Central Asia - Aral Sea, Caspian Sea and Balkhash Sea - have experienced a drastic process of desiccation in the last century. The Aral Sea is in a critical process of drying out. The area of the dry sea floor, the Aralkum, is about 46,000 km². From 1930 till 1977, the sea level of Caspian Sea declined by 3 m. The desiccated sea floor was about 50,000 km² in size and formed a new Caspian terrace. From 1973 till 1988, the sea level of Balkhash Sea declined by 2.5 m. Salt and sand deserts, salt meadows and mesophytic shrub vegetation have formed on the dry sea floors. The flora of the new coasts is an immigration flora in a stage of transformation, steady new developments and consist of about 400 species. Sedimentological and pedological factors determine vegetation and succession patterns. The typical pattern of landscapes, vegetation types and soils is striated. The actual trend in dry sea floor characteristics is the formation of the salt deserts and spreading of halophytic ecosystems, depending on the fluctuating water level. The coasts of the Aral Sea and Caspian Sea are large diversity centres of the halophyte flora and vegetation in Eurasia.

8.15.1. The 2010 challenge in ex situ conservation and threatened species recovery for botanic gardens and genetic resources communities.

P. S. Wyse Jackson¹, E. Dulloo²;
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The adoption by the world community of Target 8 of the Convention on Biological Diversity's Global Strategy for Plant Conservation, to achieve "60% of threatened plant species in accessible *ex situ* collections, preferably in the country of origin, and 10% of them included in recovery and restoration programmes" by 2010, presents significant new challenges and opportunities for the botanic garden and plant genetic resources communities. It is currently estimated that the representation of threatened plant species in *ex situ* collections is no more than 20%, and perhaps only 2% in recovery and restoration initiatives. A rejuvenated and vigorous coordinated worldwide programme in *ex situ* conservation and species recovery will therefore be required to reach this target. The roles of botanic gardens and other *ex situ* conservation centres in the implementation of Target 8 will be outlined, highlighting specific milestones identified by an international working group of relevant stakeholders in 2004 for a proposed multi-year workplan towards 2010. Proposals for monitoring progress will also be outlined.

8.15.2. Targeting of threatened species for ex situ conservation

H. H. Kolberg;
 National Botanical Research Institute, Windhoek, Namibia.

The concept of "threatened species", its various definitions, interpretations and approaches, is briefly introduced. Different terminology used and how this compares with the definition of "threatened species", is explored. Particular emphasis is placed on the IUCN system for red-listing of plants. The means to identify and subsequently prioritise and locate threatened species for seed collection for *ex situ* conservation are discussed from the perspective of a fieldworker in a developing country, Namibia. Sources of information and the difficulties in getting access to these, are mentioned. The challenges and rewards experienced in implementing such work are explained with examples. Opportunities and obstacles presented by Target 8 of the Global Strategy for Plant Conservation are briefly summarised against the background of the realities in developing countries.

8.15.3. Genetic integrity and risks in Ex Situ threatened plant collections: case studies in China

H. Huang;
 Wuhan Botanical Garden/Wuhan Institute of Botany, The Chinese Academy of Sciences, Wuhan, China.

The ultimate goal of *ex situ* conservation for threatened plants is to maintain the genetic integrity of the species and to construct a self-sustaining population with evolutionary potential for reintroduction. However, many challenges remain unsolved for *ex situ* threatened collections in botanical gardens. Because *ex situ* conserved plants in botanical gardens are unavoidably small populations, genetic weakness must not be overlooked. Lack of research for genetic related issues to *ex situ* collections has greatly hampered success of implication of such *ex situ* conserved and artificially increased plant materials to reintroduction program in wild. This presentation provides a brief review of genetic issues related to *ex situ* threatened plants growing in garden environment, including loss of genetic diversity, inbreeding depression, hybridization and introgression and adverse effects from genetic adaptation to cultivation. These genetic effects should be rigorously addressed in well designed garden experiments for a wide range of threatened plants. Several case studies for *ex situ* threatened plants in Chinese botanical gardens are discussed.

8.15.4. The role of botanic garden horticulture in the global strategy for plant conservation, with particular relevance to target 8

D. A. H. Rae;
 Royal Botanic Garden Edinburgh, Edinburgh, United Kingdom.

The Global Strategy for Plant Conservation (GSPC) contains 16 targets for plant conservation to be met by 2010 and botanic garden horticulturists can contribute to at least 8 of these. Target 8 is of particular relevance as it calls for 60% of endangered species

to be conserved *ex situ*, preferably in the country of origin, and for 10% of these to be used in recovery programmes. As a result of a survey carried out by the Royal Botanic Garden Edinburgh on behalf of PlantNetwork (the plant collections network of Britain and Ireland) it has been found that 67% of Britain's 204 red book-listed species are held as living plants in *ex situ* collections, but only a third of these are of UK origin. This project has now plotted the location of all the populations of each endangered species and has overlain this against the location of botanic gardens- the idea being to encourage botanic gardens to cultivate their locally endangered species. In this way, if each garden 'adopts' three or four species, sampled afresh from the wild, then the network of British botanic gardens will hold a devolved, networked, genetically structured collection of the country's endangered species.

8.15.5. Conservation of Chilean threatened species under the Millennium Seed Bank Project

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¹Instituto de Investigaciones Agropecuarias (INIA), Vicuña, Chile,

²Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile, ³Seed Conservation Department, Royal Botanic Gardens, Kew Wakehurst Place, United Kingdom.

The Millennium Seed Bank Project, led by the Royal Botanic Gardens Kew, is an international initiative aiming at conserving seeds from threatened plant species, principally from the drylands. Collaborative partnerships have been created worldwide and capacity for seed banking has been strengthened in-country through training, equipment and information. The Instituto de Investigaciones Agropecuarias in Chile has been working with Kew since 2002, developing a successful conservation programme centred on the endemic and threatened species from the Deserts and Mediterranean regions. Seeds from 211 endemic species including 83 threatened have been collected and conserved so far. However, the lack of updated red lists, scarce herbarium vouchers and botanical information, represent main obstacles for species targeting and conservation. Joint research will help meet recovery and restoration targets in the GSPC. Closer and more integrated collaborations between the different organizations and stakeholders involved in plant conservation would contribute to strength the national capacity for *ex situ* conservation.

8.15.6. *Ex situ* conservation - the role of plant tissue culture

M. M. Ramsay;

Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom.

Cryopreservation of living material at or near the temperature of liquid nitrogen (-196°C) is among the best methods for securing conservation collections on a long term basis as the suspension of cellular metabolic activities extends the storage life of plant material and minimizes genetic drift. Plant tissue culture techniques are valuable for recalcitrant species whose seeds cannot be stored and for taxa that are difficult to propagate by conventional means or where there are dormancy requirements to overcome. Rapid multiplication under axenic conditions can result in large numbers of shoots from small quantities of initial material and can thus provide plants for re-establishment and scientific research without endangering existing populations. Challenges to effective utilization of *in vitro* techniques to meet GSPC targets include: quantifying what is stored, where and how; assessing effectiveness; identifying training and technology needs. The value and potential of these techniques in conserving threatened plant species will be described using examples from Kew and elsewhere.

8.15.7. Re-introduction of threatened species into the wild programmes

A. N. Hitchcock;

Kirstenbosch National Botanical Garden, Cape Town, South Africa.

Kirstenbosch National Botanical Garden's *ex situ* conservation program is not carried out in isolation, but is part of a strategy, which includes *in situ* conservation. Our focus is on conserving threatened taxa from threatened habitats close to Cape Town and on the intrinsic conservation value of each collection. Priority is given to the most threatened taxa and those that lend themselves for restoration projects. The prime goal is to preserve the genetic integrity of these collections by storing them in the Millennium Seed Bank in addition to holding live living collections in our Botanical Garden. Equally important is the restoration of these key conservation areas, many of which are degraded. Partnerships

have been established with Conservation Organisations to secure these habitats from further degradation so that they remain viable for restoration projects. There are major challenges in establishing viable *ex situ* conservation collections in the Cape Floristic Kingdom. These include taxonomic and horticultural problems, fragmentation and loss of genetic reserves, limited resources and conflicting priorities within a burgeoning city environment.

8.16.1. Alpine flora and ethnobotany in Nepal: Resources vs conservation measures

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The high hills of Nepal, above 3000 m elevation, comprise 35.2 percent of the land area. Major part of its landscape is covered by high mountains and Himalayas. Approximately one third species of the flowering plants of Nepal are recorded from the sub-alpine to nival zone, of which 80% species are endemic to the Himalaya. About 64% endemic species of Nepal occur in this region. Similarly, 7.3% of the people living in this fragile habitat belong to indigenous ethnic groups of Tibetan or Mongoloid origin. Plant resources are the major source of basic commodities for the livelihood of the indigenous people residing in the remote areas. Overexploitation and haphazard harvesting of plant resources for timber, firewood, and medicinal herbs, for trade and local uses lead to deforestation and vulnerable natural habitats. Threats to plant resources are alarming where natural regeneration of commercially threatened species is extremely poor. Integrated efforts of local, national and international organizations are commendable in the conservation, sustainable harvesting and domestication of few high demand traded species in some protected and buffer zone areas of Nepal.

8.16.2. Chinese and Russian medicinal plants and their folk use.

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Ever since the first contact with the Far East, the Western world has been irresistibly fascinated by the mysterious pharmacology of China, by its promise of health, longevity, increased sexual potency, fertility, and rejuvenation. On the other hand, Western medical science continues to discover important constituents in botanicals employed in so-called "folk" medicines of various countries. As far as the folk medicine of China is concerned, the difficult and ambiguous language barrier has kept this area from being extensively studied. Many contacts in the Far East culture between Russia and China appeared to reveal a lot of similarities in pharmacology and use of various plants. We compared the medicinal plants of Russian and Chinese Far East and found many similarities as well as differences in their usage. For example, while in Russia and China *Tussilago farfara* is used as antitussive, *Inula britannica* in China is used as expectorant and stomachic, while in Russia it is mostly used for curing of skin diseases. We analyzed the use of more than 200 herbs. It is obvious that further contacts will promote to enrichment of both Russian and Chinese pharmacology.

8.16.3. Medicinal plants of Hezar Masjed Mountain chain

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Hezar Masjed, one of the two main mountain chains parallel to the direction of (Northwestern -Eastwestern) the north of Khorasan, consists of several single mountains. These sediments consist of lime, bug lime, Chile, marl, and occasionally, conglomerate and evaporative sands which each, based on the rate of hardness and other existing conditions, forms various soils. On the other hand, different height, temperature, the rain and dispersion rate, have created several ecology so that at the margin of piedmont mountain of Hezar Masjed, there is an ecology with ultra cold semiarid, at the submountain it is Mediterranean ultra cold, altitudes are with subhumid climate and at the top of mountain there is ultra cold humid climate. Generally it creates a considerable diversity in the plant cover. The medicinal plants of this area have been collected during the past years, by me and my cooperators and after detection have been stored in the herbarium of botanical research center of Ferdowsi University.

8.16.4. Native plants from the arid zones of Mexico with medicinal and pesticide potential

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Plant extracts particularly medicinal plants, are becoming increasingly important in pharmacy and have great popularity as alternative therapy. The use of synthetic pesticides creates several problems like pests resistance, resurgence, and detrimental effect on non-target organisms. Therefore, the aim of this study was to evaluate the effect of 4 plants extracts (*Larrea tridentata*, *Flourensia cernua*, *Origanum majorana* and *Bouvardia ternifolia*) from the Mexican desert on 12 phytopathogenic fungi. Also, 7 bacteria were tested for antibacterial activity, these include 5 gram-negative and 2 gram-positive bacteria. Fungi cultures were grown in potato dextrose agar (PDA) into Petri plates (Pp). Pp were incubated at 25°C. Mycelia growth was monitored by measuring the plates area covered by the fungi. Antibacterial activity of plants extracts was assayed by mixing them with Muller Hinton agar (MHA). Bacteria cultures were incubated at 36°C into 3-divided Pp. Each test was replicated at least 2 times. The four extracts analyzed showed antimicrobial activity against all organisms studied, but the *L. tridentata* extract was more active against all microorganisms.

8.16.5. Ethnobotanical studies in Ethiopia: the case of medicinal plants

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In this paper, I attempt to provide a picture of the past and current ethnobotanical studies in Ethiopia by using studies on medicinal plants as a case study. Until recently, the gathering of medicinal plants in Ethiopia was the responsibility of traditional medical practitioners. In the gathering process, spiritual, ritual, taboos, seasonal and social restrictions get high considerations. According to a recent study, Ethiopia is one of the six countries of the world where about 60% of its plants are indigenous and have a healing potential. It is believed that about 1000 types of medicinal plants are found in Ethiopia. The objectives of this paper are to identify the causes for the depletion of wild populations of medicinal plants in Ethiopia, and to explore ways for the effective conservation of all medicinal plants species in the country. It is found out that at present, the existence of medicinal plants is threatened due to the expansion of agriculture, over exploitation, deforestation, destructive harvesting and habitat alteration. The paper suggests that cultivation could be used as an alternative to over exploitation of traditional medicinal plants by gatherers.

8.16.6. Innovation and tradition in Mediterranean diet: gathered food plants

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Wild plants and weeds have been a substantial part of the diet in rural Mediterranean areas during centuries. Although it has been assumed that these types of gathered local food are the remnants of the hunter-gatherers cultures anteceding the Neolithic revolution, the ethnobotanical research brings evidence of adaptive changes in the lists of plants consumed. Recent replacements in the Mediterranean points to a resilient mechanism that allowed Mediterranean people to introduce in their diets new cultivated taxa but also weeds and invasive species. These replacements supposed changes, amongst others, in the ingest of secondary metabolites and fatty acids in the Mediterranean diet. The tender perennial ornamental *Gazania rigens* (L.) Gaertn., native from South Africa is naturalized in Mediterranean Spain. In the lower Segura basin it is now part of the gathered food plants species complex, locally known as "rampetes". The character in common to this group is the basal whorl of pinnate leaves that are collected and consumed in salads after a short boiling. Examples of "rampetes" are *Plantago coronopus* L., *Coronopus didymus* (L.) Sm. (native from South America).

8.16.7. Local food in the Mediterranean and its antioxidant effects

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Very little is known about the current role of local used food plants in the Mediterranean ('local food'). An EU-funded consortium of six academic institutions and one company (coordinated by M.H.) investigated the role of such resources in the local diets of selected regions in of the Mediterranean. For example, in the Graecanic community Galliciano (Southern Italy) about 40 wild food taxa have been identified, for example *Reichardia picroides* (used raw as a snack or cooked with other wild greens). As part of this project the broader public a booklet for usage in the region of origin has been developed. A total of 120 food species have been investigated for anti-oxidant activity using a variety of *in vitro* assays (incl. guaiacol oxidation, xanthine oxidase inhibition, HOCl scavenging, eNOS activity), and against a variety of other targets. An example is *Cynara cardunculus* ssp. *cardunculus* which shows one of the highest anti-oxidants effects in the guaiacol assay (about 95% at 0.2µg/ml).

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8.17.1. The first Eastern Central European herbals and early botanical monographs on CD-ROM and Internet

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Case studies are presented, based on E-ditions of first Eastern Central European botanical monographs, (mostly herbals): Váradi Lencsés György (1530-1593) - *Ars Medica Electronica* vol. I-VI (Budapest-Kolozsvár-Szombathely-Veszprém; 2000); Melius P. - *Herbarium about the names, nature and use of plants* (1578, Colosvar); C. Clusius - *Rariorum aliquot stirpium per Pannoniam, Austriam etc**, (1583, Antwerpen); Beythe A. - *Fives Könüv (a Hungarian Herbal, Nemetújvár 1595)*. The influence of different pharmacobotanical schools form, Sárvár (HU), Güssing/Nemetújvár (AU), and Kolozsvár/Cluj (RO) and the role of I. Sylvester, P. Melius, G. Lencsés, St. and A. Beythe - and first of all - by C. Clusius in development of botany is analysed using the new databases, including the role of Clusius in the study of Central European plant diversity and the advance of pre-Linnaean botany. An EU-7 project for botanists working in "Clusius-cities" from Austria, Belgium, England, France, Germany, Hungary, Italy, Poland, Spain and The Netherlands is outlined for education and research, to reveal hidden values of European botany by electronic methods.

8.17.2. Toward an Eastern Central European „Electronic ethnobotanical database"

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The first records about the knowledge of plants are as old as the first written relics. The diversion of vernacular and official use of plant names began in the 16th century. A lot of new plant names given by neologists of the 19th century has been drawn from traditional country use of words. The upcoming industrialisation, the extension of cultivated landscape and the changing structure of settlements caused irreversible alteration and detriment of the nature and the relation of humans to the nature were also basically changed by the new disciplines. The recent flow of population ageing process and globalization caused elementary social and economical changes of way of life. The maintenance of traditional plant varieties and land-races came to be at the end by disappearing of country life. The process described above has been demonstrated by the results of a great number of local and regional ethnobotanical studies. It is necessary to build detailed databases for the versatile landscape structure of Eastern Central Europe adjoining to world ethno- and econom-botanical databases.

8.17.3. Information literacy and botany

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We have all heard of the information explosion and the anxiety that can accompany this phenomenon. Today students at university are expected to be computer literate. They may not be information literate. The lecturers and research supervisors may not be

information literate. A manual aimed at the undergraduate biology level has been written as an information literacy tool. We need to equip new botanists with the skills to be able to *identify*, *access* and *locate* information as well as *evaluate* and *apply* information. The principles of developing a search strategy, understanding the different types of reference materials and subject materials, and being able to find information in catalogues or databases, are discussed in the manual. What changes have occurred in the information literacy level since this manual has been in use? Should information literacy be a core part of an undergraduate curriculum?

8.17.4. The study of genotype, cold pretreatment, low-dose gamma irradiation and 2,4-D concentration effect on wheat doubled haploid production

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In this study, response of a cultivar (Atrak) and two lines (F3 2005 and F3 2104) of wheat, and effect of cold pretreatment, low dose gamma irradiation and 2,4-D (2.4 mg l^{-1}) were investigated to anther culture response. Donor plants were grown under field conditions in early spring. Anthers were plated on modified CHB medium containing 2, 4-D ($2, 4 \text{ mg l}^{-1}$), 0.5 mg l^{-1} Kinetin and 90 g l^{-1} Sucrose. Percent of Callus formation in 100 anther and percent of plantlet in 100 calli were measured. Results indicated that genotype, cold pretreatment and 2,4-D concentrations had significant effect on anther culture response. F3 2005 showed highest callus induction and plantlet production and F₃ 2104 had lowest response. This experiment indicated that androgenic traits are controlled by genotype and environmental factors. Further more these traits are controlled independently.

9.1.1. Plants have effective non-host resistance against the great majority of pathogens

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All individuals of a plant species resist attack by most pathogens due to their "non-host resistance". Only a limited number of microbes are able to overcome this resistance, and can be seen as pathogens of the species. In this way non-host resistance is extremely broad-range and durable. On the other hand, host resistance, where only a subset of plant of a species exhibits resistance towards a pathogen, is often narrow in range and vulnerable for breakdown. Therefore non-host resistance is predicted to have a potential in plant disease control, provided we understand the mechanisms behind. It is conceivable that understanding non-host resistance will go hand-in-hand with understanding host resistance and the process of pathogen attack. As such it becomes clear that non-host resistance can be the combined action of a number of defence components functioning along the steps of pathogen ingress towards establishing disease. This is also true for host resistance, and it has been documented that several of these defence components are shared between the two types of resistance. But still it is unknown what in fact determines the difference between them.

9.1.2. RLKS and innate immunity in plants

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Recent studies highlight the importance of "pathogen-associated molecular patterns (PAMPs)" or "general elicitors" in basal disease resistance. One prominent PAMP is bacterial flagellin. FLS2, the receptor-like protein kinase (RLK) encoded by *FLS2* in *Arabidopsis thaliana*, functions as the receptor for flagellin. Homozygous *fls2* mutants are completely insensitive to flagellin. They are also more susceptible to *Pseudomonas syringae* DC3000 than wildtype plants, demonstrating the functional importance of this PAMP receptor in disease resistance. Another PAMP recognized by *A. thaliana* is EF-Tu, one of the most abundant proteins present in bacteria. A mutant in the *EFR* gene, which encodes an RLK as well, lacks both EF-Tu responses and binding sites for EF-Tu derived elicitors, indicating that EFR represents the functional EF-Tu receptor. A comparison of the responses of *Arabidopsis* to flagellin and EF-Tu will provide insights into the similarities and differences among the PAMP perception systems in plants.

9.1.3. Mechanisms of induced resistance against pathogens and insect attack

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After limited attack by pathogens or herbivorous insects, plants develop an enhanced capacity to defend themselves against subsequent attacks, which often occurs throughout the entire plant. Several types of systemically induced resistance have been recognized: pathogen-induced systemic acquired resistance (SAR), rhizobacteria-induced systemic resistance (ISR), and wound-induced resistance (WIR). Whereas SAR is regulated by salicylic acid, ISR and WIR are dependent on jasmonic acid; all three types also require responsiveness to ethylene. Activation of more than one type of induced resistance causes cross-talk between the different signalling pathways, allowing the plant to prioritize its defensive mechanisms. Associated with SAR and WIR additional defenses are induced, but ISR depends entirely on an enhancement of extant mechanisms to counter the invader. This "priming" phenomenon relies on the activation of either pre-existing or newly induced transcriptional regulators.

9.1.4. Conservation of disease resistance systems in two plant families

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Arabidopsis Rpm1 and soybean Rpg1b are resistance genes that mediate recognition of the AvrB protein from the plant pathogen *Pseudomonas syringae*, thereby establishing defense responses and resulting in disease resistance for the host plant. Recognition also requires the Rin4 protein in *Arabidopsis*, which interacts with both Rpm1 and AvrB and is required for Rpm1-mediated disease resistance. Recent cloning of Rpg1b has allowed phylogenetic comparison of these two functionally analogous resistance genes, which appear to have evolved independently. We aim to discover the molecular level of conservation between these two disease resistance systems from different plant families. We transformed *rpm1* mutants with Rpg1b, assayed for AvrB recognition, and recovered plants that showed resistance against *P. syringae* strains carrying AvrB. However, plants that respond to AvrB also exhibit dwarfing phenotypes. We have also identified three Rin4 homologs in soybean, and are currently assaying for their physical interaction with AvrB and their ability to functionally replace *Arabidopsis* Rin4.

9.1.5. An *Arabidopsis* glutaredoxin interacts with central components the signal transduction pathway responding to salicylic acid

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Salicylic acid (SA) and jasmonic acid are crucial internal signaling molecules needed for the induction of defense responses upon attack of pathogens. Basic leucine zipper (bZIP) transcription factors of the TGA family bind to activating sequence-1 (as-1)-like elements found in promoters of SA- and JA-inducible genes. Using a modified yeast-two-hybrid screen using AtTGA2 as a bait we have isolated a glutaredoxin (GRX28480) which is able to form a ternary complex with TGA factors and NPR1, a redox-regulated protein essential for SA-inducible gene expression. Expression of GRX28480 is induced by SA and infection with virulent and avirulent bacteria. Lines overexpressing GRX28480 exhibit reduced expression of the truncated CaMV 35S promoter, which contains an as-1 element as the only regulatory sequence. As jasmonic acid responsive genes can be repressed by SA in an NPR1-dependent manner, we are currently investigating whether GRX28480 is involved in this response.

9.1.6. Early effects of *Pseudomonas syringae* infection on primary metabolism and defence response of *Arabidopsis thaliana*

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To elucidate the effect of pathogen infection on primary metabolism and defence responses, *Arabidopsis thaliana* plants have been challenged by the bacterial pathogen *Pseudomonas syringae* pv. Tomato. Infection by a virulent and an avirulent strain of *P. syringae* resulted in a differential and complex regulation of

defence gene expression and photosynthesis. The non-invasive technique of chlorophyll fluorescence imaging revealed specific spatial and temporal effects on photosynthetic activity around the infection sites. Impairment of the photosynthetic apparatus after pathogen infection was limited to the infected tissue and could be detected prior the development of visible symptoms. Both the maximum and the effective quantum yield of the photosystem II were inhibited. The non-photochemical quenching showed a complex pattern. Northern blot analyses of representative marker genes also showed a differential response. Defence related genes were induced both by the virulent and avirulent stains although with a different time course. In contrast, the expression of the Calvin cycle gene Rubisco was only repressed in the virulent interaction.

9.2.1. Plant proteomics: Mass spectrometry driven strategies and applications

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Proteomic technologies facilitate analysis of very complex protein mixtures derived from plant cells and tissues. In this lecture, I will highlight some of the mass spectrometry driven strategies that have been employed for the study of plant proteins in our laboratory, including the analysis of membrane proteins, phosphoproteins, and quantitative studies of protein expression and interactions. Proteomic technologies, including 2D electrophoresis, multidimensional chromatography, peptide sequencing by mass spectrometry and stable isotope labeling will be discussed. Applications include proteomic analysis of GPI-anchored proteins, determination of phosphorylation sites in membrane proteins and the use of stable isotope labeling of amino acids in cell culture (SILAC) for relative quantitation of plant proteins. Approaches for de novo sequencing of peptides by tandem mass spectrometry are presented and applied to the detailed analysis of plant proteins.

9.2.2. Proteome and transcriptome characterization of *Medicago truncatula* seed development. The switch from embryogenesis to storage functions.

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Seed development is a multi-stage process requiring the coordinated expression of many genes. It starts with the conversion of the integuments into a resistant seed coat and by the formation of the endosperm and embryo. Once the embryo is differentiated, storage compounds accumulate and metabolic activities switch towards a quiescent state. As the majority of storage materials that constitute the value of seed crops are deposited during seed filling, we have investigated this period by using proteomics with *M. truncatula* adopted as a model for legumes. In this presentation, we will highlight the roles of diverse metabolic processes during seed filling. Using protein maps, we will present differential proteomics comparing embryo, endosperm and seed coat at the onset of reserve deposition. Finally, we will show data comparing proteome and transcriptome during embryogenesis, seed filling, maturation and desiccation, and also in comparisons of the seed tissues. These studies clearly identify marker genes for characteristic stages of seed development and tissue differentiation and highlight the role of various genes in the switch towards reserve accumulation.

9.2.3. Beyond big lists: building networks of interaction in the proteome of plant mitochondria for functional interpretation of a cellular organelle

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Combining isolation of mitochondria with separation techniques and peptide mass spectrometry has provided lists of >600 non-redundant genes encoding proteins that are targeted to these respiratory organelles in plants. Many challenges lie in interpreting these lists and defining the relationship between them in the operation of mitochondria. We have been determining the subset of contaminants in these sets using overlays with protein sets located elsewhere in the cell. We have been linking these protein lists on the basis of physical interactions in complexes and functional similarities by ligand affinity and also clustering their

expression during development and stress response using microarray data interpreted on the basis of proteome sets. Lastly we have been mapping, by similarity, the plant mitochondrial proteome onto the mitochondria proteomes from mammals and fungi. In this manner we learn about the evolutionary divergence of mitochondrial function, identify plant-specific classes of mitochondrial proteins and link genes of unknown function with those of known function that encode a relatively low complexity proteome set.

9.2.4. The plastid proteomes of *A. thaliana* and maize; quantitative expression profiling, protein interaction networks, functions and a plastid proteome database

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Plastids are predicted to contain several thousands of proteins, forming an interacting network of proteins. A semi-quantitative overview of stromal, as well as thylakoid-associated proteins and protein complexes in *Arabidopsis thaliana* chloroplasts and cell specific Bundle sheath (BS) and Mesophyll (M) chloroplasts will be shown. Quantitative comparative proteome analysis of purified BS and M chloroplasts was carried out by stable isotope labeling techniques, as well as 2-DE gels. Protein spots were analyzed by mass spectrometry and emphasis was placed on distinguishing between different members of gene families. Proteins were assigned to molecular functions and metabolic pathways. A Plastid Proteome Database, PPDB (<http://pdb.tc.cornell.edu/>) was created to all experimental, as well as curated and predicted plastid proteomics data, currently holding experimental data on more than 1000 plastid proteins. The database also facilitates comparison between different plastid types in maize and *Arabidopsis*. Acknowledgements: Financially supported by the NSF, the USDA and NYSTAR.

9.2.5. Membrane proteomics: Identification and subcellular localization of new transport systems

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Plastids, and especially chloroplasts, conduct vital biosynthetic functions, and many reactions are located exclusively within these unique organelles (photosynthesis, amino acids biosynthesis, fatty acids and vitamins biosynthesis...). A two-membrane system, the envelope, surrounds all plastid types and separates the plastid stroma from the cytosol. As a consequence, the envelope is involved in the controlled exchange of a variety of ions and metabolites between these two subcellular compartments. Chloroplasts also import cytoplasmically synthesized precursor proteins from the cytosol. However, only few proteins involved in these transport processes have been already identified at the molecular level. In order to identify new envelope transporters, a targeted hydrophobic and subcellular specific proteomic approach was developed to identify components of the hydrophobic core of the chloroplast envelope. We demonstrated that the development of such studies may help to reveal the hydrophobic protein composition of a particular membrane system in a given tissue, thus enhancing our understanding of the plant transport systems.

9.2.6. Proteomics of *Fagus sylvatica* L. seeds dormancy breaking and germination.

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Investigation of proteins, product of genes activated during a complex developmental process as is dormancy breakage was the aim of presented research. With seed dormancy breaking the plant hormones are associated: GA₃ responsible for stimulation of dormancy breaking and germination, and ABA responsible for maintenance of dormancy and inhibition of germination. These studies were carried out on *Fagus sylvatica* seeds during their stratification and germination. After imbibition in water (control) and in solution of GA₃ or ABA, beechnuts were subjected to cold stratification, which breaks dormancy. Regarding the proteomic approach, proteins of the seeds were separated by 2D-gel electrophoresis and were analyzed by mass spectrometry. The influence of hormones was investigated and main protein

variations were pointed out. Analysis of the proteins specific only for the GA₃ or ABA was done. The link between the variation of proteins, hormones and dormancy breaking was established.

9.2.7. Proteome analysis of differentially displayed proteins as a tool for investigating copper stress in mycorrhizal and non-mycorrhizal hemp (*Cannabis sativa*) roots.

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Due to its fast growth and capability to accumulate high amounts of metals (cadmium and chromium), *Cannabis sativa* has a role, improved by arbuscular-mycorrhizal (AM) symbiosis, in phytoremediation of metal polluted soils. A metal which is often found in polluted soils is copper, a plant micronutrient toxic at high concentrations, whose effects have not yet been investigated in hemp. The aim of this work was to investigate the response mechanisms to Cu in *C. sativa* plants grown in absence and in presence of 150 ppm of CuSO₄ and of the AM fungus *Glomus intraradices*. To evaluate protein expression pattern, analytical two-dimensional electrophoresis (2-DE) was performed on IPG strips 3-10 and 4-7 and by vertical slab 12% SDS-PAGE; 2-DE gels were Coomassie stained and analysed with the image analysis PDQuest software for qualitative and quantitative evaluation of differentially expressed proteins. For the first time we showed the 2D maps of hemp soluble root proteins and the first draft of proteins identification by nano-LC (ESI Q-TOF) MS/MS peptide sequencing of *C. sativa*, a species with unsequenced genome.

9.3.1. Pollen characteristics - achieving their potential in plant systematics and phylogeny

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By the mid 1960's light microscopy had provided a rich pollen literature, and scanning electron microscopy was becoming available commercially. Serious pollen morphologists soon appreciated its potential, because the data it produced complemented both light microscopy and transmission electron microscopy. Pioneering pollen research during the late 1960's and the 1970's saw rapid development in the application of pollen morphological data to plant systematics, phylogenetics and evolution. Many remarkable insights into pollen, as well as more routine taxonomic and systematic pollen papers, have been published, demonstrating and establishing the importance of pollen data - and some key examples are highlighted. However, as more integrated approaches to plant phylogeny take hold few institutes any longer support pollen morphology as a discipline. Therefore, if pollen data are to provide maximum value, botanists not well-acquainted with pollen morphology must acquire a better understanding, within their research projects, of the quality of previously published pollen data, as well as the appropriateness of planned pollen preparation techniques.

9.3.2. Pollen morphology and development in aquatic angiosperms

J. M. Osborn, M. L. Taylor;
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In the history of angiosperm evolution there have been numerous transitions from the terrestrial environment to the aquatic habitat. While there are many examples of aquatic genera and species nested within terrestrial families and genera, respectively, there are only about 28 families of angiosperms that primarily comprise aquatic taxa. The majority of these occur in freshwater environments, but several groups have colonized the marine habitat. Although the biology and systematic relationships of these predominately aquatic families are becoming better understood, relatively little is known about the pollen biology of these interesting plants. In this presentation, we review pollen morphology, ultrastructure, and ontogeny from the families of aquatic flowering plants for which detailed data are available, with emphasis on Cabombaceae, Nymphaeaceae, Nelumbonaceae, Callitrichaceae, and Podostemaceae. Pollen studies of aquatic angiosperms provide the opportunity to address fundamental questions about reproductive biology, ecology and evolution of pollination syndromes, evolution of key pollen characters, and phylogeny.

9.3.3. Pollen and anther ontogeny in *Brasenia* (Cabombaceae, Nymphaeales)

M. L. Taylor, J. M. Osborn;
Truman State University, Kirksville, MO, United States.

Brasenia is a monotypic water lily sporadically distributed throughout the Americas, Asia, Australia, and Africa. Evidence from a range of studies indicates that Nymphaeales are among the most primitive angiosperms. Despite their phylogenetic utility, pollen developmental characters are not well known in *Brasenia*. In this presentation, pollen and anther ontogeny will be described for *Brasenia*. Anthers at all stages of development have been studied using scanning electron, transmission electron, and light microscopy. The tetrad stage proceeds rapidly and variation in tetrad geometry has been identified. Formation of the infratectal collumellae and the tectum begins during the tetrad stage, with the tectum forming discontinuously. The endexine lamellae and foot layer form during the free microspore and early grain stages, and their development varies in the apertural and non-apertural regions of the pollen wall. *Brasenia* is wind-pollinated, and several pollen characters appear to be correlated with this pollination syndrome. The adaptive significance of these characters, in contrast to those of the fly-pollinated genus *Cabomba*, will be discussed.

9.3.4. Post-meiotic cytokinesis and pollen aperture pattern ontogeny: comparison of development in four species differing in aperture pattern

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The pollen grains of angiosperms display a wide range of variation in aperture patterns. An increasing number of studies indicate that aperture pattern ontogeny is correlated with the way in which cytokinesis that follows male meiosis is completed. To investigate this hypothesis and the nature of the mechanisms involved in pattern ontogeny, the formation of the intersporal callose walls that are formed to isolate the microspores after meiosis was studied in four species that all display different aperture patterns. Two species belong to the monocots (*Phormium tenax* and *Asphodelus albus*), the two other species belong to the eudicots (*Helleboreus foetidus* and *Protea lepidocarpodendron*). The way in which post-meiotic cytokinesis is performed differs between all four species. The variation in callose deposition could be linked to aperture pattern definition, providing new data to support the hypothesis that post-meiotic cytokinesis is involved in aperture pattern ontogeny.

9.3.5. The role of micelle self-assembly in exine development

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Exine ontogeny in *Chamaedorea microspandix* and *Trevesia spirocarpa* has been ultrastructurally studied. Our interpretation in the light of knowledge of colloidal chemistry allows to explain many steps of exine ontogeny as self-assembly of micelles, or liquid crystals. Liquid crystal systems, depending on the concentration of a dispersion, have several mesophases: spherical micelles, cylinder ones, layers of hexagonally packed cylinders (middle micelles), bilayers with water-contained gap (neat micelles), transforming one to another spontaneously. All these micelle mesophases are seen in the periplasmic space during exine development. In both species at early tetrad stage portions of the glycocalyx - glycoproteins - are seen as spherical micelles. At middle tetrad stage spherical micelles turn to cylinder ones, and then to middle micelles, corresponding to J. Rowley's tufts. The first step of the endexine lamellae development is the formation of neat micelles with water-gap (well-known white lines). In apertural oncus in *Trevesia* microspores both spherical and neat micelles are observed as granular and lamellate parts of the endexine.

9.3.6. The enigmatic pollen morphology of *Duparquetia* (Leguminosae: Caesalpinioideae)

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Phylogenetic affinities of the monotypic genus *Duparquetia* are currently poorly resolved; preliminary molecular analyses suggest a basal, isolated position among legumes. Understanding the development of *Duparquetia* pollen is necessary to deduce the

orientation of the apertures and contribute pollen characters to multi-dataset analyses. For examination of microspores within developing tetrads, we used confocal microscopy to clarify the position of the ectoapertures, and light microscopy of microspores stained with Alcian blue to locate the pores. *Duparquetia* pollen develops in a pattern similar to that of other eudicots. However, mature grains are asymmetrical with one equatorial (latitudinal) encircling ectoaperture and two circular endoapertures (pores), a feature unique in legumes and eudicots. This autapomorphic character is consistent with a pattern of greater pollen morphological diversity in basal caesalpinoid legume groups than in more derived clades.

9.3.7. Importance of pollen morphology for *Cistus* and *Halimium* (Cistaceae) systematics.

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The two genera *Cistus* and *Halimium* are shrub-like members of the family Cistaceae and occur only around the Mediterranean Sea and the Canary Islands. For this study, palynological characters have been examined for almost all species of *Cistus* and *Halimium* using light and scanning electron microscopy. Some other reproductive characters have also been investigated and are presented here. Pollen morphology is compared to a molecular phylogeny built with the plastid spacers trnS-trnG and trnL-trnF. Palynological characters as well as flower colour appear to be highly phylogenetically informative. For example, the pink flowered *Cistus* species, which share interesting microreticulate pollen exines, form a monophyletic group radiating to the Canary Islands. In the genus *Halimium*, three well defined and well supported monophyletic groups share interesting pollen characters and also biogeographical patterns of distribution.

9.4.1. Self-incompatibility and the evolution of mating systems in the Brassicaceae

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Inhibition of self-related pollen in the self-incompatibility response of the Brassicaceae is effected by allele-specific interactions between two highly polymorphic proteins, the stigma-localized S-locus receptor kinase SRK and its pollen coat-localized ligand, the S-locus cysteine-rich protein SCR. SRK and SCR variants are highly polymorphic, making it difficult to identify potential specificity determinants by sequence alignment. Modeling of 3D structures is used to identify potential specificity determinants and to understand the processes that underlie the generation of novel self-incompatibility specificities. In parallel, transgenic *A. thaliana* plants that express the self-incompatibility trait as a result of transfer of an SRK/SCR gene pair from *A. lyrata* are used for genetic screens aimed at identifying signaling intermediates and the loci required for expression of the self-incompatibility trait. Results pertaining to the diversification of the recognition repertoire and to the molecular bases of evolutionary switches from outcrossing to self-fertilizing mating system will be presented.

9.4.2. The S locus and global pollination responses among *Arabidopsis* species

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Reciprocal pollinations between self-incompatible (SI) species and self-compatible (SC) often exhibit unilateral incompatibility, whereby pollinations in which the SI species is the pistillate parent are typically incompatible, while the reciprocal pollinations are compatible. Based on these observations, which were generalized into the so-called SI X SC rule, a role for the self-incompatibility (S) locus in inter-species pollen recognition and consequently in species isolation, has been proposed. In crucifers, the SI X SC rule is applicable to the majority of inter-specific and inter-generic pollinations, including those between *A. thaliana* and its close relative *A. lyrata*. Our successful transfer of the self-incompatibility trait into *A. thaliana* by transformation with the two S-locus genes that determine self-incompatibility specificity in stigma and pollen provides new opportunities to investigate the possible contribution of the S locus to inter-specific pollen-stigma barriers. Our

observations of pollen tube growth and guidance and the ultimate outcome of these inter-specific pollinations will be presented.

9.4.3. DNA polymorphism in and around the *A. lyrata* self-incompatibility loci

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The self-incompatibility genes are highly polymorphic, both in terms of allele numbers maintained by rare-allele advantage, and in levels of diversity per nucleotide, at both synonymous and non-synonymous sites. The observations can be interpreted in the light of population genetic models for situations where selection maintains variation for certain amino acids (giving high non-synonymous site diversity), and linkage ensures high variability at nearby sites because variants arising in one allele will only slowly recombine with other alleles. This effect of balancing selection on regions of genome linked to the S-loci themselves extends to several linked loci at considerable distances away. This linkage disequilibrium is expected if recombination is suppressed in the S-locus region, as is predicted because preservation of incompatible allele combinations is necessary at the pistil recognition gene (SRK) and the gene encoding the pollen ligand.

9.4.4. Natural variation in outcrossing rates in North American populations of *Arabidopsis lyrata* (Brassicaceae)

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The genetic basis for breakdown in self-incompatibility (SI) systems is known for some groups but few studies have examined variation in selfing rates in species for which the molecular basis of SI is known. We surveyed such variation in *Arabidopsis lyrata* (Brassicaceae) in order to examine the causes and genetic consequences of changes in its breeding system. In the Great Lakes region of North America we found a surprising dichotomy in strength of SI among populations sampled from a limited geographic region. Out of 12 populations surveyed, 5 showed a preponderance of SC individuals, high levels of inbreeding (based on progeny arrays using multilocus microsatellite markers), and substantially reduced genetic diversity and observed heterozygosity compared to outcrossing populations. Geographic distributions of outcrossing and inbreeding populations are currently disjunct, suggesting that they could have originated from different source populations. Results will be discussed in terms of implications of flexibility in mating systems for adaptation in species such as this that are restricted to threatened habitats (sand dunes, alvars, cliff edges).

9.4.5. Molecular population genetics of pollen-coat genes in the self-fertilizing *Arabidopsis thaliana* and the outcrossing *Arabidopsis lyrata*

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Pollen-pistil interactions are species-specific. Genes involved in species recognition can be expected to be highly divergent between species, but have a reduced level of polymorphism within species. Levels of sequence variation in species-recognition genes should be reduced in an outcrossing as compared to selfing or apomictic species. Oleopollenin proteins are family of seven proteins that are expressed in the pollen coat of *Arabidopsis thaliana*. They may be involved in species-recognition of the male gametophyte. They evolve rapidly by gene birth and death processes and by sequence divergence. To test whether these genes are involved in species recognition, we compared levels of polymorphism and divergence in the self-fertilizing species *A. thaliana* and the self incompatible species *A. lyrata*. Sequence polymorphisms in these genes are reduced in both species relative to genome-wide levels of variation. Oleopollenins are repetitive proteins and we found a high level of variation in the number of amino acid repeats in both species. We will discuss whether observed patterns of polymorphism are consistent with a role in species recognition.

9.4.6. Reproductive and genetic consequences of diploid hybrid speciation in *Senecio*.

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Senecio squalidus (Oxford ragwort) represents an example of a recently formed homoploid hybrid species for which the parental taxa are known. The parental taxa, *S. aethnensis* and *S. chrysanthemifolius*, native to Sicily, hybridize on Mt. Etna where they have produced an extensive hybrid zone, from which the original material that gave rise to *S. squalidus* was collected and introduced to the UK approximately 300 years ago. The well-characterized hybrid history of *S. squalidus* makes it a valuable 'model' for studying the reproductive and genetic effects of homoploid speciation. We are investigating the inheritance of sporophytic self-incompatibility (SI) in wild and synthetic hybrids of *S. aethnensis* and *S. chrysanthemifolius* (both of which are strongly SI) for comparison with UK *S. squalidus* (also strongly SI). In addition, we are using anonymous cDNA microarrays to study patterns of floral gene expression in these wild and synthetic hybrids, their parental taxa and UK *S. squalidus*.

9.5.1. The green algal ancestry of land plants as revealed by the chloroplast genome

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About 500 millions years ago, freshwater green algae belonging to the class Charophyceae emerged from their aquatic environment to colonize the land. This important event in the history of our planet ultimately gave rise to all the land plant species, yet little is known about the nature of the immediate algal ancestor of land plants. We have recently undertaken the sequencing of chloroplast genomes from representatives of all five recognized lineages of the Charophyceae to elucidate the branching order of these lineages and to study the evolution of chloroplast DNA within the Streptophyta (the phylum containing the Charophyceae and land plants). We have determined thus far the complete chloroplast genome sequences of *Chaetosphaeridium* (Coleochaetales), *Chara* (Charales), *Chlorokybus* (Chlorokybales), *Mesostigma* (Mesostigmatales), *Staurastrum* (Zygnematales) and *Zygnema* (Zygnematales). We will present the streptophyte phylogenies we have inferred from multiple chloroplast gene and protein sequences as well as our comparative analyses of the gene partitioning pattern, gene content, gene structure, gene order, and intron content.

9.5.2. The mitochondrial perspective on early land plant evolution

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Most molecular studies find bryophytes paraphyletic at the base of land plants while confirming the individual monophylies of liverworts, mosses and hornworts. Mitochondrial data, notably the occurrence of introns, suggest liverworts as sister to all other embryophytes. This early dichotomy was apparently followed by differential intron invasion into the chondriomes. Further introns appear to be gained, and occasionally lost, along the backbone phylogeny of non-liverworts in genes such as *nad2*, *nad4*, *nad5* and *nad7* and include the ancestors of trans-splicing group II introns in angiosperms. Intron occurrence is congruent with a model that places hornworts as the sister group to tracheophytes. The idiosyncratic phenomenon of frequent pyrimidine exchange RNA editing in plant mitochondria is phylogenetically less conclusive. Intergenic regions, frequently investigated in chloroplast and nuclear DNA, have so far not been analysed in plant mitochondria due to a lack of conserved gene arrangements. We now find at least two gene clusters widely conserved in bryophytes as further phylogenetically informative loci.

9.5.3. Inference of land plant phylogeny from genomic chloroplast data

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The large influx of comparative DNA sequence data has led to many new inferences regarding the land plant tree of life. Early branches in the phylogenetic history of major clades are particularly hard to reconstruct due to the phenomena of long branch attraction. Several analyses spanning land plants have

used data from different genetic markers. Nevertheless, there is disagreement about the order of branching among the major clades. We have used available data from complete chloroplast DNA sequences from the plastome of 19 taxa with exemplars of charophytes, liverworts, mosses, hornworts, lycophytes, moniliforms, conifers, and angiosperms. We present several analyses of evolutionary relationships among land plants by using full nucleotide data, amino acid sequences, and by comparing gene arrangements from chloroplast genomes. The results, while still tentative pending the large number of chloroplast genomes from other key lineages that are soon to be sequenced, are intriguing in themselves, and contribute to a growing comparative database of genomic and morphological data across the green plants.

9.5.4. Phylogenetic significance and diversification of the Bryophyta sensu lato

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The earliest forms of plants on land are represented among extant lineages by the bryophytes (i.e., Anthocerotophyta, Marchantiophyta and Bryophyta). The sequence of cladogenesis following the transition to land is still fairly unresolved due to a) discrepancies in outgroup selection, b) inconsistencies in selection of ingroup exemplars as of the main lineages, c) difficulties in uncovering character sources suitable for reconstructing ancient radiations and d) recovering a phylogenetic signal that dates back nearly 500 my. Significant progress has been made in identifying the root of the mosses and the hornworts, but early divergences among the liverworts remain controversial. Character sampling has greatly expanded to multigenic and genomic data sets, but despite such intensive character samplings, inferences remain incongruent with regard to the monophyly of the bryophyta and in the case of their paraphyly, which lineage marks the earliest divergence.

9.5.5. Evolutionary history of ferns: Ancient origins and recent radiations

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By integrating molecular phylogenetic data with fossil constraints we show that by the end of the Carboniferous, all five extant fern lineages - ophioglossoids, whisk ferns, marattioids, horsetails, and leptosporangiates - were present, and the earliest divergence within crown-group leptosporangiate ferns had also occurred. The Permian witnessed the establishment of four additional leptosporangiate lineages - gleichenioids, filmy ferns, schizaeoids, and core leptosporangiates. A subsequent Triassic diversification of the core leptosporangiates gave rise to the heterosporous ferns, tree ferns, and polypods. Despite the ancient origins of all the major extant fern lineages, the bulk of the diversification within these lineages is more recent; several successive radiations since the Cretaceous generated most of modern fern diversity. Although much of the diversification occurred within polypods, which now account for more than 80% of ferns, the more recent radiations were not confined to this group. Concurrent diversifications in several other fern lineages raise important questions about the evolutionary processes underlying fern diversity.

9.5.6. Seed plant phylogenomics: Insights from multicharacter analysis and partitioning

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Phylogenetic relationships among Seed Plant groups remain controversial, even after numerous individual character sources have been used to resolve them. It is now clear that phylogenies based on single or a few molecules tend to reflect their histories, rather than that of the taxa analysed. Recent studies have supported the simultaneous analysis of multiple data partitions to overcome the effect of conflicting phylogenetic signals. A recent surge in sequencing projects (such as ours) has significantly increased the availability of molecular characters. Using a concatenation of orthologous genomic and EST sequences, with

novel bioinformatics tools and phylogenetic methods, we are addressing the issue of Seed Plant phylogeny at a multigene scale. Our aim is a fully resolved and well-supported species tree, for which individual character support can still be identified and evaluated. Results of preliminary analyses using data from several genes will be shown. Approaches to determine the extent and probable consequences of character changes, and potential implications of these analyses in expression, functional, and evo-devo studies, will be discussed.

9.5.7. Phylogenetic reconstruction and age estimation of land plants and their subclades using DNA sequences

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Origin and subsequent diversification of land plants (embryophytes) had a significant impact on evolution of life on Earth by fundamentally altering the terrestrial ecosystem. Despite a large number of morphological, paleontological, and molecular studies, several issues on land plant phylogeny remain controversial. These include: the deepest-diverging lineage of land plants, the sister group of tracheophytes, relationships among basal moniliformopses, and phylogenetic affinity of Gnetales. We have assembled a data set of eight chloroplast (*atpB*, *rbcL*, *cpSSU*, *cplSU*), mitochondrial (*atp1*, *mtLSU*), and nuclear (18S, 26S) genes from over 180 land plants and green algae. We have also gathered information on distribution of several mitochondrial group II introns and gene content in the chloroplast DNA inverted repeat. Analyses of these three data sets indicate that the evolutionary history of land plants can be reconstructed with a high level of confidence. We also use the DNA sequences to estimate the ages of land plants and their subclades.

9.6.1. Plant parasitic microfungi and their host plants in temperate and tropical areas - a comparison

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During evolution, fungi belonging to different systematic groups (mainly different orders of Asco- and Basidiomycota) adopted a plant parasitic life style. Apparently any plant can be infected by plant parasitic microfungi, but a single fungal species usually can only infect a limited range of host species. Different systematic groups of plant parasitic microfungi show primarily temperate or tropical centres of distribution according to geographical distribution centres of their host plants. Life cycles and morphological characteristics of fungi differ according to different climatic and ecological conditions. While fungi in higher latitudes are adapted to overcome winter, fungi in tropical regions have to deal with high light intensities and often quickly and drastically changing humidity and temperature values. Observations on different characteristics are presented and compared based on plant parasitic microfungi in Europe and in the Neotropics. A lack of basic morphological and systematical investigation as well as long term ecological observation and experiments on tropical plant parasitic microfungi is evident.

9.6.2. Evolution of the Proteaceae and their pathogens

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The Proteaceae are distributed predominantly in the Southern Hemisphere occurring in Australia and the South Pacific, Mexico, Central and Southern America, Africa, India and South East Asia. The foliicolous pathogens tend to be unique to these Proteaceae hosts. However, it appears that pathogens associated with Proteaceae in the various regions where members of the family occur differ, and it is possible that the divisions in the family are reflected in the host and geographic range of the foliar pathogens. In this study an attempt is made to investigate this observation using specimens collected from the field, and herbarium specimens. Pathogens of southern African Proteaceae are well understood and are observed on Australian hosts (e.g. unusual Rhyncostomatoid fungi, *Vizella interrupta* and *Verrucisporota proteacearum*). However, *Phyllachora* and other biotrophic and epiphytic taxa are common to Australian and South American Proteaceae, but noticeably lacking from southern African Proteaceae. Although patterns are emerging, more collections are necessary in Australia and South America to further elucidate fungus/host relationships.

9.6.3. Evolution of host-parasite patterns within selected Peronosporales

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In an exemplary manner, ecology, morphology, systematics and coevolution within the genus *Plasmopara* and its closest relatives is presented in the light of recent molecular and morphological investigations. Data on phylogeny, distribution, morphology and ecology of both hosts and parasites are combined to elucidate evolutionary host-parasite connections. In addition, the influence of climatic and ecological constraints (habitats) on species diversification will be debated. By comparison of host range and molecular phylogeny, the evolutionary importance of host jumps versus cospeciation is evaluated and their contribution to the currently observed biodiversity is discussed. The available molecular and morphological data on evolutive radiation of *Plasmopara* are shortly presented for the parasites of selected host families (e.g. Ranunculaceae, Apiaceae, Geraniaceae), and the importance of host specificity is evaluated. The resulting problems of species concepts (morphological versus molecular) will be briefly discussed on the basis of selected *Plasmopara* species groups.

9.6.4. Patterns and processes of host-parasite relationships in rust fungi and wild roses: integrating ecology and evolution

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Rust fungi in the genus *Phragmidium* are frequent pathogens of both wild and cultivated roses. We investigated the occurrence and relationships of rusts on dog roses, *Rosa* sect. *Caninae* (*Rosa canina*, *R. corymbifera* and *R. rubiginosa*) in Germany. Two *Phragmidium* species, *P. mucronatum* and *P. tuberculatum*, were found and they were able to infect each of the three dog rose species. However, the overall infection of *R. rubiginosa* was significantly lower. Despite overlapping host ranges, the evolutionary background of *P. tuberculatum* and *P. mucronatum* is quite distinct. Phylogenetic analyses of nuclear ribosomal DNA sequences suggest that *P. mucronatum* shares a common ancestor with other rose rusts, whereas *P. tuberculatum* evolved from a *Rubus-Sanguisorba* rust clade and must have undergone a host shift to *Rosa* spp.

The above results will be discussed in the light of recent findings concerning the evolution of dog roses and larger scale relationships of Phragmidiaceae and Rosaceae in general.

9.6.5. How can phylogenetic data contribute to the understanding of Microbotryales?

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Although the convergence of smut-like appearance of several fungal plant parasites was discussed for a long time, molecular phylogeny revealed the Microbotryales as a second independent smut group not related to the Ustilaginomycetes, but as a subgroup of the Urediniomycetes. Within the Microbotryales, characters like morphology and host species are as diverse as they are in the Ustilaginomycetes. Phylogenetic analyses of LSU rDNA sequences support a separation of Ustilentylomataceae and Microbotryaceae as predicted from ultrastructural markers. The Microbotryaceae are infectious to several plant families with a focus on Polygonaceae. The anther smuts on Caryophyllaceae represent a smaller monophyletic group. Recent data give insight into the distribution of morphological characters, the role of host specificity and joined evolution with their hosts. A comparison of the convergent Ustilaginomycetes and Microbotryales reveals differences in their host-parasite relationships through time.

9.6.6. Coevolutionary aspects in smut fungi (Ustilaginaceae) on grasses (Poaceae)

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The basidiomycete family Ustilaginaceae (Basidiomycota: Ustilaginales) encompasses about 15 genera and more than 750

species of phytopathogenic fungi. The majority of these species belong to the genera *Ustilago* and *Sporisorium*. Both genera parasitize exclusively on grasses (Poaceae) where they induce a variety of symptoms on different host organs.

Due to the highly specific occurrence of *Ustilago* and *Sporisorium* species on certain grass genera or species, the outcome of certain co-evolutionary host-parasite interaction seems to be very likely.

In order to detect nature and extent of these evolutionary interactions, we used molecular sequence data (internal transcribed spacer and large subunit ribosomal DNA) of about 50 parasite species and their hosts. Reconciliation analyses, Brooks Parsimony analyses and randomization tests of the resulting topologies will be used to discuss cospeciation events versus host jumps. These results will be compared with data from other parasitic basidiomycete genera.

9.6.7. Parasitism of rust spores by *Ramularia* species

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Three *Ramularia* species have been known as hyperparasites of rust fungi in Poland. *Ramularia coleosporii* is quite frequently found on uredial sori of *Coleosporium tussilaginis*; *Ramularia uredinis* parasitizes uredial sori of many species belonging to *Melampsora* and *Cronartium* genera, whereas *Ramularia uredinearum* is a parasite of various sori of *Puccinia* species.

Uredial and telial sori of different species of rust fungi infected with *Ramularia coleosporii*, *Ramularia uredinis* and *Ramularia uredinearum* were examined using light, scanning and transmission microscopes to determine the parasite-hyperparasite contact. The microscopical examination clearly showed that mycelium of *Ramularia* species grows on the surface of the uredial and telial sori of rust fungi and it is observed as a white coating of hyphae and conidiophores with spores. Hyphae of the hyperparasite directly penetrate rust spores causing gradually their complete destruction.

9.7.1. Progress in systematics and evolution of Bromeliaceae

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Evidence from molecular studies of the chloroplast genome is rapidly growing and demonstrating the unreliable classification of the family. While all results corroborate the monophyly of Bromelioideae and Tillandsioideae subfamilies, the opposite is true for Pitcairnioideae which disintegrates into numerous clades. However, these phylogenies have not yet been compared with data from mitochondrial and nuclear genomes. At the generic level many groups need to be reclassified. This is most obvious in the large and inhomogeneous „genera“ *Aechmea* and *Tillandsia*. Morphology of flowers, pollen, stigmata, septal nectaries, and seeds as well as anatomical characters also are in conflict with current classifications but are still insufficiently known. They do not unequivocally support the molecular data. Analyses of selected characters like CAM photosynthesis and succulence reveal numerous homoplasies reflecting extensive and rapid adaptive radiation in Bromeliaceae.

9.7.2. Phylogeny of Bromeliaceae - implications for origin, adaptive radiation, and geographic diversification

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We provide a new classification for Bromeliaceae with eight subfamilies, based on a cladistic analysis of *ndhF* sequences. Brocchinioideae is basalmost, followed by Lindmanioidae, and an unresolved trichotomy involving Hechtioideae, Tillandsioideae, and the remaining bromeliads in Navioideae, Pitcairnioideae, Puyoideae, and Bromelioideae. Bromeliads arose as C₃ terrestrials on moist infertile sites in the Guayana Shield 70 Mya, spread centripetally in the New World, and dispersed to West Africa less than 10 Mya. Modern lineages began to diverge 20 Mya and invaded drier regions beginning 15 Mya, coincident with a “bromeliad revolution” involving the repeated evolution of epiphytism, CAM photosynthesis, impounding leaves, several features of leaf/trichome anatomy, and accelerated diversification at the generic level. Epiphytism may have accelerated speciation by allowing colonization along the length of the Andes, while favoring the occupation of a dissected cloud-forest landscape. *Hechtia*, *Abromeitiella-Deuterocohnia-Dyckia-Encholirium*, and

Puya show a remarkable pattern of concerted convergence in six leaf traits adapted to drought.

9.7.3. Phylogenetics of subfamily Tillandsioideae (Bromeliaceae): a comparison of plastid and nuclear DNA sequence with morphological data

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Part of the low-copy nuclear gene phosphoribulokinase (PRK) and a multicopy nuclear rDNA spacer region (ITS) were used to clarify relationships of subfamily Tillandsioideae (Bromeliaceae). These data are compared with the available cpDNA sequences of coding *rbcl* and *matK* and noncoding *trnL* intron, *trnL-F* intergenic spacer, *atpB-rbcL* intergenic spacer, *rps16* intron, and partial 5' and 3' *trnK* intron. We added taxa of *Tillandsia*, *Guzmania* and *Vriesea* to get better resolution within these genetically convergent genera. Molecular phylogenetic data is compared with various morphological characters to test their usefulness for a new classification of Tillandsioideae. These characters are optimised onto a strict consensus tree of the combined matrices of plastid and nuclear DNA sequences. Preference has been given to characters likely to be little affected by adaptive constraints, i. e., pollen, stigma, ovules, anther, seed, and nectary morphology.

9.7.4. Improving taxa and character sampling to support generic and subgeneric units in Bromeliaceae: the example of *Alcantarea* and *Vriesea goniorachis* complex (Tillandsioideae)

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Historical difficulties encountered in defining species and generic units in Bromeliaceae have been documented on the basis of sampling related problems: loss or modification of some important structures in herbarium specimens in post-collection preparation steps and under collection and the consequent poor documentation of morphological variation in wild populations. However, recent studies using improved data mainly based on living specimens demonstrate that better taxon sampling combined with careful attention to character sampling can result in a much clearer understanding of taxonomic and phylogenetic relationships within Bromeliaceae. In Tillandsioideae, the careful selection and documentation of subutilized characters provided by living specimens strengthened the generic status of the recently resurrected *Alcantarea* and allowed a consistent delineation of the species complex headed by *Vriesea goniorachis*. Thus, besides the intensification of field activities, the establishment of well-documented living collections has a strategic importance in the conduction of new taxonomic investigation in Bromeliaceae.

9.7.5. Evolution of the inflorescence in *Puya* (Bromeliaceae): a phylogenetic point of view

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Several hypothesis can be performed about the relationship between species in Bromeliaceae. *Puya* is a predominantly South American genus subdivided in two subgenera: *Puya* (8 spp) and *Puyopsis* (187 spp). In this work, phylogenetic analyses are carried out based on morphological characters to determine if the subgenus *Puya* is monophyletic and to explore if the character-sterile branch apex of the inflorescence- is a significant character for the subgenus. We also study the evolution of other characters and their relation to pollination systems, and discuss the evolution of the inflorescence in this genus, as to shape, size and floral characters, from a phylogenetic point of view. Results indicate that petals spiraled together after anthesis and the presence of the sterile apex are synapomorphic of the genus and the subgenus *Puya* respectively, and that they are related to pollination systems.

9.7.6. Evolution of Bromelioideae (Bromeliaceae)

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We focus on subfamily Bromelioideae including 29 genera/58 species (of 40 genera and 81 species of Bromeliaceae investigated). Phylogenetic analyses of three chloroplast markers, *matK*, *trnL*-intron and *trnL-trnF* intergenic spacer, improve

resolution in Bromelioideae. *Greigia* is putative sister to the remainder of the subfamily.

The latter displays an unresolved trichotomy with branches formed by (a) *Bromelia*, (b) *Ochagavia*, *Fascicularia* and *Deinacanthon*, and (c) the remaining "core Bromelioideae".

Based on comparison of molecular phylogeny and photosynthetic pathway, life-form and biogeography, we conclude that early Bromelioideae probably had an Andean distribution, were terrestrials with C3 photosynthesis, and lacked water-impounding phytotelmata. The most derived "core Bromelioideae" are almost exclusively CAM. Additionally, the sequence data from atpB-rbcL IGS are presently included into the phylogenetic reconstruction.

9.7.7. A phylogenetic analysis of Bromelioideae (Bromeliaceae) based on molecular and morphological data

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Of the three traditionally defined subfamilies of Bromeliaceae, the systematics of Bromelioideae are perhaps the most poorly understood. A low rate of molecular evolution has made it difficult to produce a well-resolved molecular phylogeny. Likewise, morphological data have proved difficult to apply due to a high degree of morphological variation, lack of preserved floral and fruit material, and the difficulty of obtaining field-collected materials. Bayesian and parsimony analyses of morphological and molecular (*trnL-trnF*, *psbA-trnH*, and *matK* sequence) data were conducted to resolve intra- and intergeneric relationships within the subfamily. An emphasis on floral and fruit characters has yielded numerous new phylogenetically informative characters. Analysis of each data set (morphological and molecular) separately generated large numbers of trees (> 10,000) with moderate resolution and support. Combining the data sets, however, yielded far fewer trees and greatly increased resolution and internal support. Numerous genera (e.g. *Aechmea*, *Portea*, *Araeococcus*) are highly polyphyletic, and a restructuring of generic circumscriptions is needed.

9.8.1. Dispersal and establishment of ground layer mosses and Lichens: dynamics at the local scale

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Bryophytes and lichens dominate the ground layer in conifer-dominated forests. The dominant lichens, *Cladonia mitis* and *C. rangiferina*, form a mosaic of extensive patches intermixed with the feather mosses *Pleurozium schreberi* and *Hylocomium splendens*. We studied lichen/moss responses to canopy opening 7 years post-thinning. Our 4 questions were posed around determining the responses to canopy opening of 1) vegetation, 2) diversity, 3) diaspores, and 4) establishment. Conclusions are that canopy opening has effected ground layer vegetation, but has had little effect on overall species richness. Both lichen and moss diaspores are abundant. Moss vegetation has decreased, yet lichens have not yet recolonized the resulting non-vegetated space. We conclude that lichens are generalists and with time are able to establish under a variety of environmental conditions, however they are inhibited by pre-existing substrates. Feather mosses are specialists and they are limited by environmental conditions inhibiting their establishment, except in local areas where pre-existing substrates and canopy conditions have precluded lichen establishment.

9.8.2. Local scale genetic structure of bryophytes

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Genetic variation in nature is structured at multiple scales. We describe the structure of genetic variation and phylogenetic relationships in mosses and liverworts at relatively local scales ranging from among-individuals within populations to among-populations within a particular geographic region. In contrast to traditional views of bryophytes as being largely clonal, genetic analyses over the last 25 years have revealed high levels of genetic diversity at local scales. Genetic/phylogenetic analyses at these scales provide insights into the relative importance of sexual and asexual reproduction in natural populations, gene flow patterns, natural selection, and interspecific hybridization. We also compare insights from different molecular approaches and

evaluate the strengths and weaknesses of alternative methods. The applications of genetic and phylogenetic studies to conservation biology of bryophytes are reviewed. Finally, we suggest directions that should be given priority in future research.

9.8.3. Influence of forest modification on epiphytic bryophyte diversity in the tropics

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The dynamics of epiphytic bryophyte assemblages along disturbance gradients have rarely been investigated in the tropics. We sampled epiphytic bryophytes in primary and recovering secondary montane forests and on remnant trees in Costa Rica and Ecuador. Species richness values in primary and secondary forests did not differ significantly, showing that old-growth primary forests are not necessarily more diverse than secondary ones. Diversity on remnant trees, however, was significantly reduced. Canopy closure and atmospheric humidity were important parameters influencing bryophyte diversity along the disturbance gradient. Shade epiphytes and projecting life forms were most strongly affected by the environmental changes. Similarity in species composition of secondary and primary forests increased with forest age, but after 40 years of succession about one fourth to one third of the primary forest species had not re-established in secondary forest. Community composition in primary and secondary forests also differed markedly, indicating that long time is needed for re-establishment of bryophyte species and communities in the regenerating niches.

9.8.4. How does life history attributes shape local scale genetic structure?

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In this study three dioicous species are compared; *Sphagnum angermanicum*, *S. fuscum* and *Pogonatum dentatum*, to see how differences in life history and ecology can be reflected in genetic structure within populations. The difference is mainly in frequency of sexual reproduction; *S. angermanicum* rarely sporulates, *S. fuscum* often have sporophytes and in *P. dentatum* sporophytes are common and numerous. This will affect the rate of recombination and together with differences in establishment rates shape the genetic structure. None of the species have specialised asexual reproduction but they occupy habitats of different longevity. Asexually formed clones may grow large in stable environments, reducing the risk of being eliminated by stochastic events and increasing sexual reproduction by formation of contact zones between sexes. In contrast, unstable environments rarely host large clones. Population level genetic structure data supports a trend from large uniform clones in the rarely reproducing species *S. angermanicum* to many small clones in the frequently reproducing *P. dentatum*.

9.8.5. Matrix population models - an unused tool among the bryologists

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Matrix population models have been used as an important tool in vascular plant and animal population biology for more than 20 years, addressing questions that otherwise are hard or impossible to analyse in a quantitative way, e.g. the lifetime impacts of herbivory and the effects of various kinds of disturbance. Matrix population models are also used in population viability analyses and is an important tool in population management studies. Bryologists have however been very slow to adopt these models, probably because of the general small size of bryophytes making tagging and tracking of individuals/ramets for following their fate hard or even impossible. In this presentation I will give a short introduction to the life cycle graph, linking the individual and the population, and the simplest matrix population model, the deterministic linear model. I will then go into the particular challenges associated with application on bryophytes before I exemplify the potential matrix models may have for bryologists.

9.8.6. Sexual reproduction in unisexual bryophytes - novel insights and implications

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Fifty to 60% of mosses and a slightly higher fraction of liverworts are unisexual. Despite an expected 1 female: 1 male progeny sex ratio at meiosis, female-dominated sex expression is the general pattern. More than $\frac{2}{3}$ of 103 taxa scored exhibit a female-biased sex ratio. Spatial segregation of sexes and skewed sex ratios affect the chance for sexual reproduction. Fertilization success in two pleurocarpous mosses is shown to be distance-dependent and limited by mate availability. Maximum fertilization ranges are several times larger than previous estimates. In *Dicranum polysetum* sporophyte formation incurs a cost in terms of reduced future growth and sex induction frequency. In the female-biased and rarely sporophytes bearing *Pseudocalliergon trifarium*, annual gametangial investment is larger in females than males, and gametangia formation does not affect vegetative growth. Thus, higher reproductive costs in males than in females cannot generally account for male rarity in unisexual mosses.

9.8.7. The role of sexual and asexual reproduction for local dynamics and rarity

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Formation of propagules is important for the survival of any species. Sexually produced propagules (spores) have a dual role as they also contribute to genetic diversification in addition to being able to disperse efficiently. Asexually produced diaspores do not contribute to the genetic differentiation (except for somatic mutations) but are important for the dispersal. As asexual diaspores are generally larger than spores they are assumed to contribute more to local dispersal and survival of populations while the smaller spores are assumed to be more important for distance dispersal. The frequency and size of spores and propagules in the liverwort family Lophoziaaceae is analyzed in relation to rarity (distribution ranges and local population sizes) and the local dynamics. The latter is related to habitat persistence, i.e. how often a species need to move to a new locality/substrate patch due to disappearance of suitable substrate.

9.9.1. Phylogeny and biogeography of the angiosperm family Rubiaceae

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Robbrecht's world survey, using trends to define subfamilies, was the last of an era. Simultaneously the first molecular analysis saw the light. The numerous such contributions are reviewed. Only Antirheoideae proved to be polyphyletic; the other subfamilies and many tribal concepts ± withstood the test of molecular phylogeny. Rubioideae were confirmed almost unchanged. Ixoroideae (s.lat.) and Cinchonoideae (s.str.) needed mutual re-delimitation, but can no longer be defined morphologically. At lower levels too, (partial) answers to long-standing disputes were given, e.g. on the question if Psychotria needs splitting.

(1) The position of *Luculia* and *Coptosapelta*, isolated at basal position in most of the former results, is re-analysed based on five chloroplast and three nuclear genes in order to examine the root of the Rubiaceae tree. (2) Published plastid phylogenies allow the construction of a supertree for more than 500 species in 300 genera, based on *rbcL*, *atpB-rbcL* spacer, *rps16* and *trnL-F*. (3) A DIVA analysis of the geographical distribution of extant terminal taxa allows to reconstruct potential ancestral distributions.

9.9.2. Diversity and biogeography of the Mesoamerican Rubiaceae

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For study of origins of diversity in Rubiaceae, the native Mesoamerican Rubiaceae present a well documented case. Mesoamerica, the region comprising Chiapas, Mexico through Panama, is varied ecologically and physiographically with a complex geological history. Many highlands originated as islands, and the varying ages of these and adjacent regions require a more

complex interpretation than areas of strictly continental origin. The Mesoamerican Rubiaceae comprise 804 species in 109 genera. 96 genera belong to 25 tribes; 13 genera are incertae sedis as to tribe. 8 genera are endemic to the region: of these 5 belong to Hamelleae, 1 to Sipaneeae, 1 to Hillieae and 2 incertae sedis for tribe. 351 species (44%) are endemic to this region, indicating significant diversification within it. Most of the endemic genera are from geologically older areas and habitats, while most of the endemic species are from younger ones. The continental Guianas have 60% as much area, 64% as many genera (77% shared), 2 endemic genera, and 50% as many species. High diversity of Rubiaceae is expected in other geologically complex regions, e.g. southeastern Asia and the Pacific Islands.

9.9.3. Evolution of myrmecophytism in Naucleaeae s.l. (Cinchonoideae, Rubiaceae)

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Myrmecophytism occurs in 140 of the 11 000 species, 22 of the 630 genera, and 11 tribes of Rubiaceae. These rubiaceae myrmecophytic species are predominantly distributed in Southeast Asia, with comparatively few species in mainland Africa and the Neotropics. They produce different types of myrmecophytic traits that provide nesting space exploited by distantly related ant species. Evolution of myrmecophytism within the mostly paleotropical tribe Naucleaeae s.l. and its most speciose genus *Neonauclea* s.s. is tested using molecular-based phylogenies. Our analyses strongly show that myrmecophytism has evolved independently at least two or three times within Naucleaeae. They also support multiple origins of the Bornean myrmecophytic *Neonauclea* but perceive no support for either the hypothesis of a single origin of the Bornean myrmecophytic *Neonauclea* or that of monophyletic origin of the non-myrmecophytic *Neonauclea*. We conclude that the uniform myrmecophytic traits in the Bornean myrmecophytic *Neonauclea* have evolved through parallel evolution and therefore are likely to mislead morphological-based phylogenies if included in analyses.

9.9.4. Dating the Rubiaceae phylogeny

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Rubiaceae is the fifth largest angiosperm family with over 10,000 species. We present a phylogeny with molecular dating of the family, including the three subfamilies, Rubioideae, Cinchonoideae, and Ixoroideae and a majority of the tribes. To get the underlying tree for dating, we performed different phylogenetic analyses of a maximum of 540 taxa and five different molecular markers (two protein-coding genes and three from non-coding regions). Branch lengths were estimated using maximum likelihood (GTR+G model), and dating used Bayesian and penalized likelihood methods. We used two different approaches when calibrating the tree. In the first, we accepted an estimate of 78 MYR for the stem lineage of Rubiaceae from an earlier analysis of the asterids as a whole. The second approach used available fossil evidence from within the Rubiaceae for calibration. All three subfamilies are of Palaeocene origin. The Ixoroideae tribes mainly diversified during Eocene and Oligocene and the Cinchonoideae and Rubioideae tribes diversified also during Miocene.

9.9.5. The circumscription of the Coffeaeae (Rubiaceae) based on nuclear and plastid sequence data

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The tribe Coffeaeae (Rubiaceae) was formerly restricted to two genera: *Coffea* and *Psilanthus*. Recent molecular studies (Andreasen & Bremer 2000, *Amer. J. Bot.* 87: 1731-1748; Persoon 2000, *Nordic J. Bot.* 20: 257-269.) infer that members of tribe Gardenieae subtribe Diplosporinae (viz. *Bertiara*, *Diplospora*, *Tricalysia*) should be removed from Gardenieae and placed in Coffeaeae. Plastid sequences (*trnL* intron, *trnL-F* IGS, RPL 16 and *accD-psa1* regions) and nuclear sequences (ITS region) of the above genera and four other members of Gardenieae subtribe Diplosporinae (viz. *Argocoffeopsis*, *Calycosiphonia*, *Discospermum*, *Sericanthe*) support an enlarged concept for the

Coffeae. *Belonophora*, a genus unplaced within the Rubiaceae tribal system, is also considered to be a member of the Coffeae. On the basis of the molecular and morphological evidence *Bertiera* is excluded from the Coffeae.

9.9.6. Phylogenetic analysis of the subfamily Ixoroideae (Rubiaceae), with a focus on the controversial systematic position of some genera

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The subfamily Ixoroideae (Rubiaceae) includes many genera that exhibit secondary pollen presentation. Recent phylogenetic studies have resolved three major clades within the subfamily. One clade includes mainly the Ixoroideae sensu Bremekamp, the other two clades include genera that were traditionally placed in the subfamily Cinchonoideae. We have expanded the sampling to include more Palearctic members of the subfamily and tested the unresolved placement of *Dialypetalanthus*, *Platycarpum*, *Posoqueria*, and *Retiniphyllum*. A phylogenetic analysis of the *trnL-F* spacer and *rps16* intron confirmed the recent delimitation of the tribes Mussaendeae, Pavetteae, and Octotropideae and reject the inclusion of *Scyphiphora* in the Ixoreae. *Dialypetalanthus* is in a clade of subfamily Cinchonoideae genera. The other clade containing Cinchonoideae genera also includes *Platycarpum* and *Posoqueria*. *Retiniphyllum*, the single member of the tribe Retiniphylleae, is sister to a clade that comprises the Ixoroideae sensu Bremekamp. We are continuing to expand the sampling inside the tribes Sipaneae, Henriquezieae, Posoquerieae, and Retiniphylleae.

9.9.7. Phylogenetic signal in morphological data

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Molecular revolution has markedly increased the number of systematic DNA-based phylogenetic studies. In contrast, morphological systematic studies have diminished and have even been discarded with the arguments that those matrices contain a high degree of homoplasy and are unreliable. We re-examine the value of organismic data even though it may contain homoplasy, using the Rubiaceae family as example. Parsimony analyses of more than 450 terminals with representatives of the main lineages of the Rubiaceae were performed. Analyses based on single DNA regions or on combined datasets yielded similar results at higher hierarchical levels, retaining three main clades. Organismic data, such as crystals, aluminum inclusions, pollen, flower and fruit characters corroborate these clades and others less inclusive. Combined analyses of molecular and morphological datasets show that morphology contains significant phylogenetic signal. Morphology facilitates the circumscription of diagnosable groups and allows the use of reciprocal illumination to (re-) examine cases of homoplasy.

9.10.1. Asexuality, polyploidy and the male function

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Polyploidy influences many aspects of asexuality in cosexual plants. These include: the expression of asexuality; the potential for reproducing sexually via the male function; the extent to which asexuals can coexist with related sexuals; and the geographic range over which asexuals are distributed. These influences will be examined. Detailed consideration will be given to the interaction between polyploidy and the male function in apomictic flowering plants and in asexual ferns. In apomictic flowering plants, polyploidy can cause disturbances during the reductional male meiosis. This can profoundly reduce the efficiency of the male function, which can increase the length of time apomicts and their sexual relatives coexist. In asexual ferns, male gametes are produced by processes that retain the full (sporophytic) chromosome number. As a result, crosses between related sexuals and asexuals result in offspring having a higher ploidy

level than their asexual parent. This polyploidisation effect of the male function of asexuals can decrease the length of time asexuals and their sexual relatives coexist.

9.10.2. Apomixis may evolve by reticulate evolution and appears to be stabilized by asexuality or structural heterozygosity

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Gametophytic apomixis involves destabilizations of the megasporocyte or nucellar cells, the egg, and the central cell or polar nuclei. This is accompanied by the heterochronic elements of apomixis: unreduced embryo sac formation, parthenogenesis, and autonomous or pseudogamous endosperm formation. We discovered that onset timing of each major germline sequence (GS) stage is independently regulated by heterochrony gene(s) and that apomixis can be induced by uncoupling GS stages through reticulation-based breeding. Apomixis may have evolved in nature in a similar manner. In coadapted sexual ecotypes, systems of heterochrony genes responsible for timing of GS stages function harmoniously; but in certain reticulation-derived derivatives of hybridization, these gene systems compete causing intermediacy between sexuality and sterility with apomictic embryo sac formation, parthenogenesis, and autonomous or pseudogamous endosperm formation arising. For apomixis to persist in nature, the causal allelic combinations, from multiple loci, must be genetically stabilized. This is accomplished through sexual sterility or karyotypic heterozygosity.

9.10.3. Genetic diversity and reproductive biology in Italian populations of the facultative apomict *Hypericum perforatum* L.

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Fingerprinting methods based on multi-locus DNA markers were used to determine the genetic diversity within and relatedness among landraces of St. John's wort. Five out of 15 sampled populations were grouped apart from the main cluster of populations revealing substantial levels of genetic differentiation. All populations resulted polyclonal and characterised by distinct rates of multi-locus genotypes. Flow cytometric analysis of both bulked and single seeds revealed variation in ploidy and suggested facultative apomixis for all populations. Cytogenetic analysis showed that sexual and aposporic processes can initiate within the same ovule but only rarely sexual and aposporic embryo sacs are present together. Moreover, the egg cell is in an active metabolic state before pollination and likely its parthenogenetic activation to form an embryo occurs before fertilization and endosperm initiation. Comparison of cyto-histological and molecular data leads to the conclusion that natural selection effectively acts in favouring genotypes with an prevalent apomictic mode of reproduction so preserving the most locally adapted gene combinations.

9.10.4. Genetic diversity within and among Kentucky bluegrass (*Poa pratensis* L.) worldwide accessions

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Kentucky bluegrass (*Poa pratensis*) is a hardy, persistent, attractive forage and turf grass adapted to a wide range of soils and climate. Its mode of reproduction is extremely versatile and ranges from naturally obligate apomixis to complete sexuality. Because different plants may have contrasting modes of reproduction, *P. pratensis* could serve as a model species for investigating apomixis and its inheritance. We are currently investigating both candidate genes for apomixis and the evolution of the apomictic trait. Here we report the characterization of 33 accessions of Kentucky bluegrass collected in 24 countries. Most of the accessions (25) represent wild material whereas the other 8 are either the most cultivated varieties (such as, Banff, Midnight, and Princeton) or the most used for Kentucky bluegrass breeding (e.g. Nugget). Thirty plants per accession were grown and are maintained to study both morphological traits over a period of three years and the ploidy level through evaluation of DNA content. DNA was extracted from each genotype and analyzed by SSR markers, chloroplast SNPs and by a candidate gene-derived SCAR marker.

9.10.5. Genetic diversity and the dynamic nature of facultative apomictic complexes: the case of *Ranunculus auricomus*

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Sources and effects of genetic diversity in agamic complexes remain largely unexplained. The *Ranunculus auricomus* complex, comprising c. 600 allopolyploid, aposporous and pseudogamous agamospecies plus four di- to hexaploid sexual species, provides a model system for study. Isozyme, AFLP and SSR analyses revealed a high genetic diversity and divergence of sexual taxa; agamospecies harbour considerable genotypic variation within and among populations, which usually consist of predominant clones and a few deviating genotypes. Character compatibility analyses showed that genetic variation within apomicts is caused to a high extent by facultative recombination. Backcrossing of apomicts to sexual species, and hybridization between agamospecies contributes only locally to genetic variation of apomicts because of spatial and ecological differentiation. Apomicts occupy a broader range of habitats and a much larger distribution area than sexuals. The complex originated probably from wide crosses resulting in an initial pool of diverse asexual genotypes, and expanded via facultative sexuality and ecological differentiation of lineages.

9.10.6. Non-random patterns of hybridization, polyploidy, and B chromosomes in the *Boechera holboellii* complex: possible insights into apomixis origins?

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The *Boechera holboellii* complex comprises *B. holboellii*, *B. divaricata* and *B. stricta*, members of which can reproduce through sex or apomixis. Sexuals are diploid, whereas apomictic individuals can be diploid or triploid, with or without B and aneuploid chromosomes. B chromosome carriers and triploids have originated via hybridization events between genetically and geographically divergent members of this group. Diploid plants containing the Bs reproduce apomictically, suggesting that the supernumerary elements are associated with apomixis. Repeated origins of B chromosomes and polyploidy imply that the apomictic phenotype has been expressed multiple times during the evolution of this complex. Analyses of single nucleotide polymorphisms (SNP) and microsatellites show that apomictic *Boechera* are characterized by similar levels of genetic variability compared to sexuals. The nature and genomic distribution of this variability will be discussed in light of the possible mechanisms which may have led to apomixis expression.

9.10.7. The costs and benefits of sexual and asexual reproduction in *Taraxacum*.

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In this paper I review studies of the cost of sex in natural dandelion populations (*Taraxacum officinale*). The cost of sex is largely a theoretical concept. The real costs of sex in nature depend basically on the biology of the system and its natural context. Over the last couple of years we have measured gene flow in mixed sexual-apomictic dandelion populations. We also have studied the heritability of apomixis. This allows us to estimate the cost of meiosis in the field. To evaluate these data for the long-term stability of mixed populations, we have integrated these data in computer models. Low triploid pollen fertility and non-transmission apomixis genes via haploid pollen transmission facilitate the coexistence of diploid sexuals and triploid apomicts dramatically. It is shown that tetraploids can play an important role in the generation of new asexuals, even although they are rare in mixed populations. Data show that sexuals experience an important cost of non-fertilized ovules in certain years with bad weather conditions during flowering period. Other costs of sex are discussed.

9.11.1. Aquatic primary production in artificial environments

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Artificial aquatic environments are of growing importance. Water management measures in rivers and lakes have tremendous impact on the availability of light and nutrients for the primary

producers. Eutrophicated lakes are often restored by means of destratification via bubbling plums which change nearly all environmental parameter like temperature, light climate and biological networks. Additionally, artificial new environments have been formed by flooding mining areas in different areas in the world. In some cases the resulting lakes contain extremely acidic water bodies up to values of 1.5. The efficiency of photosynthesis is of great importance in all these different ecotypes. In nutrient rich manipulated waters the efficiency of biomass formation should be as low as possible, whereas in new lakes from former coal mining photosynthesis should contribute as much as possible to the alkalisation. The symposium gives an overview on the conditions for primary production in these artificial waters and reports how the basic processes of biomass synthesis can be regulated on a physiological level.

9.11.2. Acidic Lakes: a challenge for aquatic botany and bioremediation.

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Acidic waters show a very broad range of acidity and pH, from rain-acidified soft water lakes (pH 4-5.5) to geogenically acidic mining waters (pH 2.5-3) and extremely acidic volcanic lakes and rivers (till pH 0). The numbers of species living in these extreme systems decrease with lower pH. In the extremely acidic waters the algal species found were largely identical: *Chlamydomonas acidophila*, *Keratococcus* (*Chlorella*) *raphidioides*, *Euglena mutabilis*, and *Ochromonas* sp.. Biomass maxima were found as high as in eutrophic neutral lakes. In a nutrient rich acidic lake, growth rates and biomasses of a nearly monospecific phytoplankton community were measured continuously by automatic sampling, and growth reached rates as determined under laboratory conditions. Mixotrophy was important in these species to different degrees, showing both types, osmotrophy and phagotrophy. The phototrophic and mixotrophic algae, both grazed by Heliozoa (*Actinophrys* sol) as the top carnivore of the food chain, showed a stratification of physiological types, explaining the deep chlorophyll maxima (DCM) found in different pit lakes and in volcanic lakes.

9.11.3. Interactions between photosynthesis, respiration and nutrient acquisition in algae

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Inorganic nutrient acquisition by algae usually occurs against a concentration gradient so is an energy demanding process, often with direct involvement of photosynthetic energy pathways. Assimilation of inorganic nutrients, particularly nitrogen, also requires the supply of C skeletons from photosynthesis or from intermediates of respiratory pathways. Conversely, inorganic nutrients are essential for maintenance of energy transduction systems. This interdependence results in complex interactions between nutrient uptake and assimilation processes and photosynthetic and respiratory energy metabolism. In this paper, we review 1) the basic processes by which algae take up nutrients such as C, N and P, 2) the energy requirements for the uptake and assimilation of these nutrients and 3) what is known about the biochemical interactions between energy metabolism and nutrient acquisition. We will discuss the consequences of nutrient uptake and assimilation to rates of respiration and photosynthesis and how these can be investigated. We also show how these interactions can be exploited in assessing nutrient status of algal cells in the environment.

9.11.4. A possible phosphorus limitation in *Chlamydomonas acidophila* Negro living in a low pH environment

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Chlamydomonas acidophila, a dominant phytoplankton species in the very acidic Lake 111 (pH 2.65) faces low concentrations of inorganic carbon (C_i), phosphate (P_i) and potassium (K⁺). In an integrated laboratory and field investigation, the potential (co-)limiting factors operating within this lake were explored. Measurements of phosphatase activity, threshold concentrations of soluble reactive phosphorus (SRP_i) and C:P ratios all suggested that the growth of this alga was potentially limited by P_i. Analysis of

field samples suggested that phosphatase activity did not result from a complexation of P_i with either metals or organic carbon. Furthermore, the cellular P_i demand in *C. acidophila* was similar to those in other *Chlamydomonas* species. Low K^+ concentrations resulted in higher minimum phosphorus quotas (Q_0) and higher SRP, than in P_i -limiting medium. Moreover, low K^+ concentrations decreased the cellular chlorophyll a content and possibly explain the low primary productivity rates measured in Lake 111. A relationship between CO_2 concentration and Q_0 was observed under K^+/P_i -limited conditions, but not under P_i -limited growth.

9.11.5. Photoprotection in phytoplankton: regulation and ecophysiology

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The photosynthetic efficiency and productivity of phytoplankton depend on the light environment which can be highly variable in aquatic ecosystems. Fluctuating irradiances due to water motions and especially excessive light exposure can be harmful for the photosynthetic apparatus. In order to cope with fluctuating light intensities, phytoplankton organisms have developed various mechanisms for their photoprotection. They safely dissipate, 'deviate', store and recycle excess energy from light absorption. Among these mechanisms, the dissipation of excess energy (or NPQ, non-photochemical chlorophyll fluorescence quenching) is considered as one of the most important mechanisms on the short time scale (seconds and minutes). New results will be presented: 1) on the regulation of NPQ in diatoms and cyanobacteria, and compared to land plants, 2) on the potential involvement of photoprotection in the spatial distribution (coastal, open ocean, etc) of species in relation to the turbulence of waters, and consequently the range and rapidity of light fluctuations they are submitted to.

9.11.6. Photoacclimation processes in phytoplankton: Mechanisms, consequences and applications

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In nature and bioreactors phytoplankton are exposed to fluctuations in light intensity. These include seasonal and diel changes in irradiance, and those stemming from vertical mixing over the light field. In algal mass cultures and photobioreactors similar changes take place as cultures grow denser, and as cells are mixed in the system. In order to survive supraoptimal, free radical generating irradiance levels as well as exposure to dim light phytoplankton species are capable of photoacclimation. Light harvesting pigments increase under low light, decreasing under high light, resulting in cells being rather transparent under strong light all the way to optically black under limiting irradiance levels. These processes are complemented up to five fold changes in Rubisco per PSU levels, resulting in parallel changes in light-saturated photosynthetic rates. Photoacclimation can be used to optimise biomass and target product yields in biotechnological applications.

9.11.7. Photoautotrophic growth under acidic conditions: A physiological comparison of two *Chlamydomonas* species

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Mining lakes in central Germany are characterized by low pH-values (2.6 - 3) and high cFetot (3 μ M). *Chlamydomonas acidophila* (*C. a.*) isolated from an acidic lake was physiologically compared to the wild type strain *Chlamydomonas reinhardtii* (*C. r.*). Growth rates of *C. a.* at pH 2.65 were similar to those of *C. r.* at pH 7. Both algae showed almost the same carotenoid composition, whereas Chl a/b ratio is twice as high in *C. a.* as in *C. r.*. The photosynthetic performance was investigated with PAM-, fast fluorescence and oxygen evolution measurements. Both species have similar quantum yield and photosynthetic capacities under growth conditions. Fluorescence induction curves indicate an increased reduction state of the PQ-pool in the dark, fast transitions from state 2 to state 1 at low light and a stable state 2 at high light. These effects were more pronounced in *C. a.*. In both strains the fluorescence based electron transport rates do not match the oxygen evolution rates on the basis of absorbed quanta. As a conclusion, we propose that both species operate with alternative electron cycling and that it is an adaptive mechanism in an acidic environment for *C. a.*

9.12.1. New perspectives on the role of Plasmodesmata and Raffinose-family Oligosaccharides in phloem loading

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The extremely high hydrostatic pressure in the phloem of most higher plants results from an energetic concentration of soluble carbohydrates in the sieve elements and companion cells (phloem loading). Loading in the minor veins of leaves occurs by two species-specific mechanisms. One involves plasma membrane transporters (apoplastic loading) while the other involves plasmodesmata (symplastic loading). It has been postulated that plants using these alternate mechanisms can be distinguished on the basis of plasmodesmatal frequencies. However, studies on several species with abundant minor vein plasmodesmata indicate that they load via the apoplast. Symplastic loading appears to be associated exclusively with the transport of raffinose-family oligosaccharides (RFOs) by polymer trapping. Species that do not translocate these sugars load via the apoplast, no matter how abundant their minor vein plasmodesmata. We suggest that the adaptive significance of RFOs in phloem transport lies exclusively in their role as polymer trapping agents. Symplastic loading will be discussed from ecological and evolutionary perspectives.

9.12.2. Reduction in nucleotide-sugar supply for cell wall polymers cause changes during seedling development

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Arabidopsis primary cell walls contain large amounts of hemicelluloses and pectic polymers. Roughly 50% of the cell wall biomass is synthesized from the nucleotide sugar UDP-glucuronic acid, the precursor for arabinose, xylose and galacturonic acid. Two pathways contribute to the synthesis of UDP-glucuronic acid from photosynthesis assimilates, each being controlled by a small gene family in *Arabidopsis*. The entry key enzymes are UDP-glucose dehydrogenase or inositol oxygenase in the second pathway, respectively. Both reactions lead to an irreversible oxidation of their substrates. Thereby the nucleotide sugar pool used for the synthesis of storage compounds is separated from the pool for cell wall polymers. Knock-out mutants in single genes lead to viable plants with almost normal phenotype. However, a more detailed analysis revealed changes in individual mutants, effecting root growth, cotyledons or flower development. The changes are specific for particular isoforms indicating an individual role for each member of the gene family. The phenotypes of knock-out mutants and aspect of pathway cross-talk will be discussed.

9.12.3. Soluble sugars and starch in leaves of congeneric savanna and gallery forest trees

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The central plains of Brazil are occupied by a complex of seasonal savannas and gallery forests, known locally as "cerrado". In the cerrado, the radiation of woody plants into savanna and forest species has occurred independently in a large number of genera. In this study, we compared the soluble sugars and starch concentrations in leaves of eight congeneric pairs of savanna and gallery forest trees in the wet and dry season. Forest species showed higher concentrations of both starch and soluble sugars. However, starch concentrations were significantly higher in the wet season while soluble sugars were higher in the dry period. This result suggests a reduction of starch by low water availability and therefore to a drop in the supply of carbon compounds in the leaves during the dry season. Sucrose was the most abundant soluble sugar. Pinitol was detected only in five out of the 16 species and had higher amounts in the dry season, when light interception by evergreen trees is much higher. In leaves, pinitol is considered to play a role as an antioxidant. Thus, this increase in the dry season may be related to photoprotection. CAPES, CNPq, FINATEC.

9.12.4. Phloem loading in two Scrophulariaceae species: what can drive symplastic flow via plasmodesmata?

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In order to determine the driving forces for symplastic sugar flux between mesophyll and phloem, gradients of carbohydrate concentrations and of osmotic pressure were studied in leaf tissues of *Alonsoa meridionalis* and *Asarina barclaiana*. In *A. meridionalis*, phloem loading is symplastic as indicated by intermediary companion cells in the minor vein phloem. In *A. barclaiana*, two types of companion cells, modified intermediary cells and transfer cells, occur in minor vein phloem, indicating a complex loading. We identified all phloem-transported carbohydrates in both species and analyzed the levels of carbohydrates in chloroplasts, vacuoles and cytoplasm of mesophyll cells. Osmotic pressures in single cells, whole leaves and phloem saps were compared. The data show that diffusion of sugars along their concentration gradients cannot be the major mechanism for symplastic phloem loading in these species. We conclude that in both plants, apoplastic phloem loading is an indispensable mechanism, and symplastic entrance of solutes into the phloem may occur by mass flow. The conditions favoring symplastic mass flow from mesophyll into the phloem are discussed.

9.12.5. Import of glucose 6-phosphate into amyloplasts and its impact on potato tuber starch content

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Amyloplasts depend on the import of carbon and energy to synthesize starch. Carbon enters the amyloplasts in form of glucose 6-phosphate via the glucose 6-phosphate/phosphate translocator (GPT), and energy is imported as ATP via the adenylate translocator (NTT). We analyzed the impact of decreasing or increasing glucose 6-phosphate import into amyloplasts of potato tubers on starch synthesis and composition as well as tuber yield. Following an RNAi strategy GPT activity was decreased, on the other hand plants were created that simultaneously overexpressed pea GPT and Arabidopsis NTT to increase the corresponding transport activities. Whereas an increase of both carbon and energy import into tuber amyloplasts led to increased tuber starch content and yield, decreasing carbon (and also energy) import led to opposite effects. These findings suggest that carbon and energy import into tuber amyloplasts co-limit starch production and tuber yield of potato plants.

9.12.6. Role of quercitol in drought stress adaptation of eucalypts

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The occurrence of the cyclic polyol quercitol among certain eucalypt species correlates strongly with the distribution of these eucalypts in arid environments. Yet, the function of quercitol in stress adaptation to low internal and external water potentials is unknown. We investigated the role of quercitol as a stress metabolite in a glasshouse experiment containing 13 eucalyptus species and a field study with *Eucalyptus astringens*. Seedlings of all species investigated contained quercitol and it occurred in leaves, branches, stems and roots of these species. Quercitol concentration decreased during the growth of well-watered plants and drought stressed plants of "mesic" species, whereas it was maintained or increased in droughted "xeric" species in the glasshouse experiment. In "xeric" species in the glasshouse and in *E. astringens* in the field tissue concentrations of quercitol increased after drought exposure (up to 300 mmol kg DW) and contributed to an overall increase in leaf osmolality. Quercitol played a vital role in the osmotic adjustment and contributed to around 20% of the total osmotic potential of *E. astringens* in all plant organs.

9.12.7. Leaf metabolites provide functional links between physiology and evolution in the genus *Eucalyptus*

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Increasing aridity is universally regarded as a major factor in the evolution of the Australian vegetation. The genus *Eucalyptus* contains more than 700 species that collectively represent a broad

ecological spectrum. *Eucalyptus* spp. constitute the dominant stratum among a range of ecological types, from the wet sclerophyll forests characteristic of the south east of the continent, to the woodlands of the dry interior where rainfall may be as little as 250 mm per annum. Targeted metabolite profiling has, for the first time, uncovered a quantitative yet discrete biochemical link with eucalypt taxonomy and evolution. Here I present a broad, metabolite-based analysis of the genus, targeting soluble carbohydrates and polyols, that is then discussed in relation to adaptation of *Eucalyptus* spp. to aridity. In particular, the distribution of cyclic polyols (cyclitols) among *Eucalyptus* species, and their proposed functions in plant tissues, provides putative links with the acclimation of trees to arid environments.

9.13.1. Metabolomics: a key technology for systems biology

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What is the function of a gene? For all approaches to this question the characterization of the phenotype is the key. A phenotype can be characterized morphologically, chemically (metabolomics) and biochemically (proteomics). Metabolomics aims at the identification and quantification of all metabolites (metabolome) in an organism, proteomics gives a view of the proteins.

One can use a targeted approach for selected compounds, or a non-targeted approach in which as many as possible compounds are measured. In both cases one can aim at the identification of all compounds or only those that show variation. Methods applied are: chromatography (LC, GC), mass- (MS) and NMR-spectrometry. Each has limitations. Chromatographic methods and MS are most sensitive, in NMR all compounds can be quantified with a single internal standard. Reproducibility of NMR is best. Chromatographic methods are dependent on the columns. No methods fulfill all demands for the ultimate goal: measure all metabolites. NMR gives a macroscopic view of the metabolome, the other methods may be used for specific groups of metabolites. Metabolomics is a major tool for systems biology.

9.13.2. Metabolic Characterization of Phytochemicals and their Metabolic Effects

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Metabonomics combines sophisticated analytical tools such as NMR spectroscopy and mass spectrometry with multivariate statistical analysis to generate complex metabolic profiles of plant and animal tissues, fluids and cell extracts. The technology provides a systems approach to measuring dynamic biochemical responses of organisms to biological stimuli and operates by profiling the metabolic responses of key intermediary biochemical pathways. Such analysis generates information regarding the metabolic status of organisms, metabolic phenotypes of genetically modified organisms and enables prediction of a range of pathological conditions. Various chemometric strategies for optimizing the characterization and interpretation of complex spectra can be adopted to increase the sensitivity of metabonomic analysis and to accommodate the dynamic nature of living systems. Applications in plant biology include quality control of plant products and characterization of growth/ageing, stress responses, geographical origin and growth conditions. In addition the technology is explored as a tool for profiling metabolic consequences of phytochemicals.

9.13.3. Multidisciplinary pattern recognition: a tool to find proper medicinal plants within the genus *Achillea*

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The genus *Achillea* includes about 130 species, i. a. "*A. millefolium* L.", which is the required source of "*Millefolii Herba*" in the European Pharmacopoeia. A content of not less than 0.02% chamazulene is requested. This is confusing, as *A. millefolium* L. s.str. is now more narrowly defined as a hexaploid species which lacks proazulenes, the precursors of chamazulene. Therefore, we searched for pharmaceutically more appropriate members within the species group of *A. millefolium* agg. which includes about 20-30 different, diploid (2x) to polyploid (4x, 6x and 8x) taxa throughout the N. Hemisphere. For this purpose, many populations (each with 5-50 individuals) from about 30 taxa from *Achillea* and *A.*

millefolium agg. were screened with multidisciplinary methods: morphometrics, anatomy, phytochemistry (GC, GC-MS, TLC), cytogenetics and DNA-analyses (ITS and trnL-F sequences, AFLP, plastid haplotypes). Our results show that the *A. millefolium* agg. is the hybrid linked crown group of the genus. As a polyploid complex it exhibits an enormous spectrum of diversity which is recombined in various ways in its species, of which some correspond to medicinal requirements.

9.13.4. Correlative GC/TOF/MS based metabolite profiling and LC/MS based protein profiling reveal time-related systemic regulation of metabolite-protein networks and improve pattern recognition for multiple biomarker selection

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A novel approach is presented combining quantitative metabolite and protein data and multivariate statistics for the analysis of time-related regulatory effects of plant metabolism at a systems level. For comparison, leaves of wild type plants and starchless mutant plants deficient in phosphoglucomutase activity (PGM) were sampled at intervals throughout the day/night cycle. Using principal and independent component analysis, each data set (metabolites and proteins) displayed discrete characteristics. The integrated metabolite/protein data set resulted in an improved ability to distinguish between WT and PGM plants (first independent component) and, in parallel, to see diurnal variations in both plants (second independent component). The described method is applicable to all kinds of biological systems and enables the unbiased identification of biomarkers embedded in correlative metabolite-protein networks [1, 2]. [1] Weckwerth, W., Wenzel, K., Fiehn, O., *Proteomics* 2004. [2] Morgenthal, Wienkoop, S., Scholz, M., Selbig, J., Weckwerth, W., *Metabolomics* 2005.

9.13.5. Metabolic profiling of root and shoot-induced changes in *Brassica nigra* (Brassicaceae).

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Plants produce a wide variety of chemical compounds as defences against the many phytophages in their natural environment. Many of these compounds are inducible and their levels will change after the plant has been damaged. Interactions between induced defences have been studied extensively for aboveground (AG) herbivores, but there is increasing evidence that root feeders (BG) induce systemic responses that affect aboveground phytophages, and *vice versa*. These AG-BG interactions may have a significant impact on how induced defences function and evolve in natural environments. We used GC MS and 2D NMR analyses to study the metabolic state of *Brassica nigra* plants after shoot or root induction with jasmonic acid (JA). Our data show that JA-application induces changes in both primary and secondary metabolite levels. Furthermore, plants induced with JA to the roots show a different metabolic profile than shoot-induced plants. We will discuss metabolic profiling as a technique to help us unravel interactions between root and shoot-induced defences. Eventually, we will link these interactions to plant and phytophage performance in both lab and field conditions.

9.13.6. Analysis of *Hypericum perforatum* (St. John's Wort) lots by NMR based metabonomics

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Hypericum perforatum L. (Clusiaceae), also known as St. John's wort, is one of the best characterized phytotherapeutics on the market. A broad variety of secondary metabolites (e.g. phloroglucinols, flavonoids, naphthodianthrones) have been characterized and their pharmacological activities have been evaluated. Within the last years, addressing biological diversity and metabolic time courses have become major topics of NMR based metabonomics. This methodological approach does combine 1D proton NMR spectroscopy with chemometrical data processing. We applied this methodology to *H. perforatum* drug

batches of different origin serving as test case sample for investigating secondary metabolite profiles of phytopharmaceuticals. A principal component analysis (PCA) carried out with 600 MHz 1D ¹H-NMR spectra allowed to discriminate the lots unambiguously. The correlation of the obtained differentiation with specific spectral regions was facilitated by the loading plots. The obtained results are compared to HPLC-DAD/MS derived metabolic profiles obtained from the identical samples.

9.14.1. Vegetation survey: moving to larger scales

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Survey research of plant community diversity across landscapes has a long tradition, particularly in continental Europe where phytosociological vegetation classification has been a popular occupation of plant ecologists throughout the past century. Currently there is an increasing demand in many parts of the world for vegetation surveys over large areas to be used for habitat inventory, monitoring and setting conservation priorities. With the increasing availability of information technologies such as database management software, multivariate statistical methods and geographic information systems, the focus of vegetation survey is shifting from local to large scales. Two major trends can be recognized. In the countries with a long tradition of vegetation sampling, many thousands of vegetation plot records were stored in electronic databases and revisions of previous vegetation typologies, using formalized methods, were prepared. In other countries, where survey research is newly introduced, stratified sampling plans designed with GIS are used, which minimize the sampling effort in the field and maximize the representativeness of the data.

9.14.2. Constancy values depend on plot size - a problem for vegetation classification and how it can be solved

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Most approaches to vegetation classification are based on the comparison of constancy values between different units. As species richness is a function of plot size, constancy values must be, too. In phytosociology, nevertheless, most authors use varying plot sizes as long as these exceed the so-called 'minimal area'. However, from a statistical point-of-view it is not correct either to calculate constancies for plots with varying size or to compare these between units sampled on different areas. Moreover, classification results crucially depend on the used plot sizes ('spatial grain'). Thus, recently several proposals have been made to standardise relevé areas for future studies. However, the question remains how to make use of the millions of 'old' data. Based on literature and own data from different vegetation types, I analysed whether the increase of constancies with increasing plot size follows a certain 'law'. It can well be described by a function of the form $C(A) = 1 - (1 - C[A_0])^{(A/A_0)^k}$ with C being the constancy, A the area and k a parameter. I present values of k for several plant communities and test the validity of extrapolations by use of this function.

9.14.3. Vegetation inventory and mapping of Taiwan

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Taiwan has a great diversity in natural forests ranging from subalpine thickets to tropical rain forests. Although the flora, vegetation formations and principal forest types are well known, there is no detailed vegetation classification based on floristic composition available. It is determined to map the natural vegetation in five years to the scale 1:5000 even the accessibility in many regions is difficult and the orthophotos are the only available images. The classical approach is modified with GIS technology. The delineating polygons are classified based on the corresponding environmental types, which are created by GIS. Sampling sites then chosen according to the combined analyses of vegetation polygons and environmental types. Finally aero and ground surveys are taken to evaluate polygon homogeneity, to

make releve sampling, and to refine the polygon attributes. All survey data, including old vegetation survey data, are stored in Taiwan Vegetation Information System, which will be used to establish a national vegetation classification system based on sampling data analyses, and to map the distribution of community types.

9.14.4. The U.S. national park system vegetation characterization and mapping program

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This paper describes the origins, protocols, standards, and status of the 10 year old USGS/NPS Vegetation Characterization and Mapping Program (<http://biology.usgs.gov/npsveg/>). Experiences in using the U.S. National Vegetation Classification System developed through cooperative efforts of the Federal Geographic Data Committee, The Ecological Society of America, NatureServe, and the National Park Service and U.S. Geological Survey will be described. Currently under review and revision, the NVCS, classifying existing vegetation, is a 7 tier hierarchical schema nesting general physiognomic types with detailed floristic types. The Program has thus far identified, described, quantified, classified and mapped hundreds of distinct plant association types within the 24 parks completed. Over 100 more parks widely distributed across the nation's biomes and ecosystems are currently under study. Many applications of the digital vegetation data have been developed to support park management. Three new protocols dealing with small parks, sparse vegetation types, and fire fuels data collection for fire behavior models are also presented.

9.14.5. 'Synthetic floras' - a flexible tool in large scale vegetation analysis

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The concept of synthetic floras offers a wide range of applications in large scale vegetation sciences. A synthetic flora comprises all species occurring at a given locality or grid cell and is derived from overlapping species distribution maps. Consequently, a synthetic flora list reflects rather the potential than the real vegetation of a locality. The compilation of large grid data sets of synthetic floras allows to analyse large scale spatial (e.g. continental) patterns of biodiversity, of vegetation composition and of the respective controlling environmental factors. Furthermore, these grid based synthetic floras can be used to classify vegetation with regard to a multitude of characteristics such as morphologic, physiologic and ecologic traits. The concept and use of synthetic floras is illustrated for an European data set of woody angiosperms covering 108 species and 5166 (0.5° x 0.5° lat./long.) terrestrial grid cells.

9.14.6. Is vegetation classification a natural or an artificial classification?

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So-called artificial classifications generally use only few attributes of the objects to be classified, are designed for pragmatic purposes and are directly applicable, e.g. separating eatable from poisonous plants.

In contrast to this 'natural' classifications (including vegetation classification) are general-purpose classifications of high predictive value, which are destined to represent causal relationships between the objects. They shall arrange objects so that a maximum of correlation can be found in each class. Furtheron generation of hypotheses shall be facilitated and, after all, the classification should allow scientific progress. The criterion for a 'good' natural classification is not to approach 'reality' but to produce useful classes for defined purposes (such as just mentioned) which can be proved by being widely used in scientific communities or in everyday life. In my contribution the logical relationship of phytosociological classification to artificial and natural classifications and the resulting consequences for syntaxonomy shall be explained.

9.15.1. Narrow endemism as a top priority in taxonomy and conservation (a Crimean example)

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The vascular flora of the Crimean peninsula (Ukraine) was a model for studying endemism during 150 years, with assessments varying from 12 to 279 species endemic to Crimea. I reconsidered Crimean endemism to 127 species, and it fits well the phytogeographical gradients. The study showed again a pressing need to overcome disagreements and parochialism for making data on endemism more realistic and comparable. Defining endemism, I recognize chorionomic and scaled approaches. When combining them, narrow endemics can be referred to areas not exceeding e. g. Sicily, Peloponnesus, or Crimea. Such areas correspond to one of the IUCN criteria for endangered and vulnerable taxa so that wider ranges refer to lower risk taxa. Each country is primarily responsible for preserving its narrow endemics, even those yet to be reconsidered taxonomically and phytogeographically. This will help avoiding the "agony of choice", misselected taxa, resource dispersing, and overfilling of Red Lists. Because of their monotypic origin, all species were sometime narrow endemics. That is why the latter should be considered as a key but fragile potential for further plant cover evolution.

9.15.2. Endemism, rarity, and reintroduction: A case study in *Silene* (Caryophyllaceae)

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Endemism arises for multiple reasons, from anthropogenic habitat alteration to range extension into newly created landscapes. Today botanists are increasingly faced with enhancing native populations at risk for extinction. Thus, reintroduction of rare species into historically-occupied habitats is a tactic growing in popularity: it simultaneously increases population size and diversifies gene pools. A 20-yr case study of rare vs common *Silene douglasii* (Caryophyllaceae) illustrates the factors affecting rarity in herbaceous perennials and the potential means of population recovery. Demographic analysis shows that inbreeding, seedling establishment, and loss of pollinators all contribute to rarity and recovery in rare var *oraria*, extant only in 3 sites. Despite similar ex situ germination, seed reintroduction produced significantly fewer juveniles of *oraria* than common var *douglasii*, and outbred transplants had higher survival and reproduction. Nocturnal moth pollinators also visit only common vars *douglasii* and *rupinae* indicating key roles for pollination and genetics in crafting effective restoration strategies for endemic plants.

9.15.3. *Allium* L. sect. *Codonoprasum reichenb.* (Alliaceae), a taxonomic and chorological study: what is a taxonomic value of narrow endemics?

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Allium subgen. *Allium* (Alliaceae) is evolutionary the youngest subgenus of the genus. Taxonomy of A. sect. *Codonoprasum* Reichenb. is complicated, because species limits are not consistent, a lot of infraspecific taxa unsuitable for modern *Allium* taxonomy were described, and identification of herbarium specimens is unreliable. Some widely spread species of the section form local derivatives, which are being described as narrow endemics: about 10 new species were described during past 15 years, though the revision of the whole section was not submitted. According our revision the section includes 68 species (and 7 subspecies) of total 750 species in the genus. Species of the section are widely spread in Europe, North Africa and Middle East. Territory of Greece and Turkey is the main centre of species diversity with high level of endemics. The species of the section look rather uniformly, though there are some species, which are intermediate in habit between A. sect. *Codonoprasum* and A. sect. *Scorodon* C. Koch. The following evolutionary trends were determined provisionally: spring blooming autumn blooming, umbels without bulbils umbels with bulbils.

9.15.4. Distribution patterns and conservation measures of local endemic *Centaurea* species in Turkey

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Compositae represented by the highest number of species and subspecies is the largest family within Turkey. *Centaurea* genus of this family has an exceptional position among the other genera and also in Flora of Turkey. It is the third richest genus in terms of the number of species and the endemic species, following *Astragalus* and *Verbascum* genera. According to the Turkish Flora *Centaurea* genus comprises 184 species, 111 of which are endemic. The endemism ratio is 62 % with 142 endemic taxa. Besides some widespread endemic species, some are only restricted to a few localities. In this presentation, distribution patterns of narrow endemics in CR and EN categories of Red Data Book and their relation with the ecological factors were explained. Additionally, the conservation measures taken for the threatened or endangered endemic species were also stated.

9.15.5. Narrow endemism and its bearing on conservation in the floras of Madagascar and New Caledonia

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The exceptionally diverse floras of Madagascar (c. 12-13,000 spp.) and New Caledonia (c. 3,300 spp.) are renowned for their high rates of endemism at the species level (c. 90% and c. 76%, respectively) and also at higher ranks. A large portion of the endemic species on both of these ancient continental islands have highly restricted geographic distributions and/or occur at a limited number of sites, and thus qualify as narrow endemics. Recent studies have shown that species ranges on both islands are strongly correlated with physical and eco-geographic parameters (e.g., bioclimate, substrate, elevation) whose complex spatial distribution largely accounts for the high proportion of narrow endemics. We examine some of these correlations, provide examples of factors that may have generated local centers of endemism, and discuss the concepts of Extent of Occurrence, Area of Occupancy, range-size rarity (endemism richness), and irreplaceability in relation to narrow endemism, and the critical role narrow endemics must play in conservation planning.

9.15.6. Recovering narrow endemic plant populations on Trindade Island, Brazil

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Vegetation recovery on Trindade, a Brazilian oceanic Island, was monitored from 1994 to 2004, during and immediately after the deliberate eradication of feral goats. The colonization of entirely barren volcanic tuffs by the narrow endemic sedges *Cyperus atlanticus* Hemsl and *Bulbostylis nesiotis* (Hemsl.) C.B. Clarke, and the native fern *Pityrogramma calomelanos* (L.) Link measured on ten permanent quadrats, achieved 80% cover in 3 years. Another narrow endemic, currently with thousands of individuals regenerating from a seed bank, *Plantago trinitatis* Rahn was considered extinct until goat populations were considerably reduced by 1998. Known only from the type collection in 1965 and described as extinct, *Peperomia beckeri* E. F. Guimarães & R. J. V. Alves is yet to be rediscovered. The status of endemic plant populations and vegetation cover of Trindade Island is correlated to the estimated populations of feral goats. All interventions which lead to this successful recovery are explained and commented in a historical perspective.

9.16.1. Beyond the tower of babylon: Taxonomy and ethnobotany in Central Asia

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The mountains of Central Asia, Tibet and the Himalayas are of great significance to the evolution and diversity of the northern temperate flora. Indigenous knowledge of plants and their uses, in great depth and detail, has a long tradition in Asia and is alive in many communities. However, this cultural heritage is endangered by recent developments and assessing the biological diversity of Central Asia remains a huge task. This paper presents results of

taxonomic work in the genera *Codonopsis* (Campanulaceae), *Leontopodium* (Asteraceae), *Oxytropis* (Fabaceae) and *Rhodiola* (Crassulaceae), which challenge some issues connecting basic and applied research. Botanical taxonomy and nomenclature are important means of communication and remain the basis for various fields of research including phylogeny, vegetation sciences and ethnobotany. Collaborative and integrative studies may help to conserve and sustainably develop natural and cultural resources, while also maintaining inter-cultural exchange in a globalizing world.

9.16.2. Medicinal plants of Mongolia

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A brief overview over the history of research on the Mongolian flora is given. The large Mongolian territory is mostly mountainous and covers different geographical zones characterised by an extreme continental climate and by a unique vegetation. Three main vegetation zones - forest-taiga, steppe and desert - as well as transitional zones, such as mountain forest steppe and semi-desert, are developed. In Mongolia, around 3000 species of vascular plants, belonging to 662 genera and 128 families are registered. During the last 30 years expeditions to all areas of the country were made, and by studying about 25000 specimens, 584 species were newly detected. The Mongolian flora is rich in medicinal plants and about 1000 species are effectively used in traditional Mongolian medicine. As a result of my studies on the resources and distribution of medicinal plants, a monograph including photos of 227 species of widely used medicinal plants was published in 2003.

9.16.3. Medicinal plants in Tibetan botanical treatises and medical works

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This paper will give a brief survey from a tibetological perspective of the botanical treatises by Tibetan authors that until now was not done yet. They will be compared with Western publications that make use of some of these sources. It will be shown that surprisingly the latter employ a monolithic concept of Tibetan medicine. They give statements that are actually stripped of any data that reflect the historical, regional and sectarian variance as found in Tibetan written sources. These data are, however, indispensable to a serious assessment of the curing potentials of medicinal plants in as far as they are the opinion of Tibetan doctors. Moreover it will be emphasized that it is necessary to recheck statements given in Tibetan botanical treatises with regard to medical works. It will be illustrated by several case studies of medicinal plants that the curing potentials as stated by Tibetan botanical works (and henceforth Western authors who made use of a limited number of them) do not match the Tibetan medical treatises. By this it will be underlined that an interdisciplinary approach that includes tibetological research can significantly add to botanical studies.

9.16.4. Ritual plants for healing, medicinal plants for trade - Ethnobotany among the Nanman-Xumu (Hengduan Mountains, SW China)

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The Hengduan Mountains are remarkable for both their biological diversity and their cultural richness. They are spanning SW Sichuan, NW Yunnan and part of eastern Tibet. For several thousands of years, the area has been inhabited by a large number of ethnic groups with distinct and different languages, customs, and land management practices. The area is also a rich source of traditional Chinese and Tibetan medicinal plants, the bulk of which is still harvested from the wild. The Nanman-Xumu are a Tibeto-Burman ethnic group living exclusively in the Shuiluo Valley, a distinct geographical region in the south of Hengduan Mountains. They do collect medicinal plants for trade but mainly use ritual plants for healing purposes. Results from eight months of ethnobotanical field research among the Nanman-Xumu are presented. The use of ritual healing plants both in daily life and by ritual specialists versus the use of medicinal plants are emphasized. The relationship between rituals, landscape and environment is discussed.

9.16.5. Variation in use and knowledge of the medicinal moss HuiXinCao

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HuiXinCao (*Rhodobryum giganteum*) is a common medicinal plant in Yunnan, China used to treat heart ailments. It can be purchased in many different places, ranging from airport souvenir shops to herb dealers in the village market. This medicinal is a moss, which is interesting because mosses generally do not play an important role in the affairs of people. HuiXinCao is also interesting because it is used in different cultural contexts. It is collected, sold, and used by minority groups of Yunnan as well as Han. In addition, it has been studied for its pharmaceutical properties in pharmaceutical factories in Shanghai. The examination of this moss across these different cultural contexts indicates the ways that the ethnobotanical knowledge about a particular plant can vary, and the possible ways that this knowledge might change over time as a result of cultural interaction. Here I discuss peculiarities of HuiXinCao that arise because it is a moss, some of the ways that knowledge about HuiXinCao varies, and how studies of ethnobotanical variation can contribute to studies of ethnobotany in general.

9.16.6. Seabuckthorn-programme for mustang, Nepal: Safeguarding medicinal plants and the biodiversity of Nepal

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As one measure to safeguard medicinal plants and the biodiversity of Nepal and as a sustainable income source for local people, HimalAsia has started in Mustang, Nepal, to establish four seabuckthorn-nurseries. This programme was initiated in cooperation with two community based cooperatives and with two *Amchi* families (doctors of Tibetan medicine). It was as well planned to begin to harvest the wild growing seabuckthorn forests that are found in certain areas of Mustang in a sustainable way: The collection from wild forests for the species of seabuckthorn *Hippophae tibetana* and *Hippophae salicifolia* in Mustang has started in autumn 2004 by forming women biodiversity forest consumer groups, mainly from low income groups, who were trained by HimalAsia to carry out this work without overharvesting the wild forest. The basic idea is to establish more seabuckthorn nurseries/seabuckthorn forests in different communities of Upper and Southern Mustang in Nepal to use it as an income source for the locally involved actors, and to show that this approach could serve as a model that can be replicated in other areas of Nepal.

9.16.7. Conservation issues of Paanchaunle (*Dactylorhiza hatagirea*) Plant in Nepal: An experience from the Annapurna Conservation Area, Lamjung

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Paanchaunle (*Dactylorhiza hatagirea*) is one of banned species of high altitude with high market demand and values. Due to its ban on harvest and transport the local inhabitants are in the opinion that its conservation will not be economic incentives to them. A study was carried out at Annapurna Conservation Area of Lamjung district, Nepal between 1997-1998 focusing mostly on socio-economic and biophysical aspects, in justifying the further need of its ban. Information was also collected on existing market prices, marketing channels, etc. from primary and secondary sources. The study concludes that both seeds and vegetative methods between 2500-5000m altitude could propagate it. Root tubers mature after two years in natural conditions and plantations that are best collected during Sept-October. These tubers get decay if they do not harvest by two growing seasons. The study area could produce annually as much as 100.00 kg. of air dry tuber worth of value US\$ 70 000 in a sustainable way that could contribute a lot in reducing the poverty at resource available sites. Local inhabitants are expected to motivate from such income for long-term conservation of the resources.

10.1.1. Interrelationship of amphimixis and apomixis

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Apomixis and sexual reproduction (amphimixis) are closely interrelated. A better understanding of the genetic and molecular basis of sexual reproduction will provide new tools towards the engineering of apomixis in sexual crops. We have used *Arabidopsis* and maize as model systems to identify genes that play a role in key steps of sexual reproduction, which may also function during apomixis. We concentrate on three developmental processes that are highly relevant to the engineering of apomixis: (1) megasporogenesis (apomeiosis), (2) fertilization (parthenogenesis), and (3) endosperm initiation (functional endosperm). By screening through a few thousand enhancer detector lines we identified genes expressed in specific cell types such as the nucellar region where for instance aposporic initials form in apomictic species, the megaspore mother cell, the egg cell and central cell. The use of these genes and their promoters for mis-expression studies and comparative analyses will be discussed.

10.1.2. The discovery of parthenogenesis: a long journey to the truth!

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1839/41, a voucher of John Smith, Kew, says „Notice of a plant which produces perfect seeds without any apparent action of pollenâ€œ. This plant, *Coelebogryne ilicifolia*, and the phenomenon of parthenogenesis in general, were strongly debated in the following years. According to Strasburger (1877), parthenogenesis did not exist at all in angiosperms. Only Juel (1898/1900) increased the insights into the subject, especially by showing the relationship between the changes of the haploid and diploid phases of the life cycle, thereby laying the basis of present day apomixis research. The discovery of parthenogenesis was made obstructed by many preconceived ideas and a lot of work was required to demonstrate this fascinating phenomenon. This talk will review the history of early apomixis research and explore the long and arduous journey it took to make parthenogenesis in plants an accepted fact.

10.1.3. Apomixis- preliminary lessons from *Poa*, *Hypericum* and wheat

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Poa pratensis has been used to study the inheritance of apomixis. The data support a model with five major genes required to control asexual seed formation: apospory initiator and preventer, parthenogenesis initiator and preventer and a megaspore development gene. Differences in expressivity and interactions of these genes are responsible for the wide variation of the mode of reproduction. Apospory and parthenogenesis segregated independently. In *Hypericum* controlled crosses between a completely sexual diploid and an obligate apomictic tetraploid plant led to the isolation of a putative apospory-related AFLP-marker. A first characterisation of putative *Arabidopsis* homologs will be reported. The Salmon system of wheat comprises three isogenic alloplasmic lines with either zygotic or autonomous, fertilisation independent embryo development. cDNA libraries of sexual and parthenogenetic egg cells have been used for molecular subtraction and EST sequencing. Egg cell specific candidate genes have been isolated. Putative *Arabidopsis* homologs have been identified and will be discussed.

10.1.4. Genes linked with apomixis: identification and characterization

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Pennisetum squamulatum Fresen and *Cenchrus ciliaris* L. are two related apomicts in the grass family. They reproduce primarily by apospory where four-nucleate embryo sacs are formed from nucellar cells. The unreduced egg develops by parthenogenesis while endosperm development requires fertilization of the central cell. Although apomixis in these two species segregates in

Mendelian fashion, a large, non-recombining portion of a chromosome is transmitted implying that multiple genes necessary for apomixis could reside in this linkage block. Physical mapping of parts of the chromosomal region transmitted with apomixis has been conducted and has shown that considerable macrosynteny exists between the two apomictic species throughout the region. Genes contained within bacterial artificial chromosome (BAC) clones have been identified and their DNA sequences have been used to determine the extent of macro- and micro-synteny with rice. Although the apomixis-associated region appears highly heterochromatic, several of the genes from this region are transcribed when tested by RT-PCR for apomict-specific transcripts.

10.1.5. Physical mapping of apomixis in *Paspalum simplex* and candidate gene discovery

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Apomixis is a natural mode of reproduction that would allow fixation on seeds of a desired genotype if introgressed in valuable crops. Several efforts are underway to reveal the genetic control of the character. In our model *Paspalum simplex*, as in other aposporous species, genetic mapping via molecular markers (AFLPs, RFLPs) revealed a block of recombination, a partial hemizigosity and a strong synteny of EST markers with the telomeric end of the long arm of rice chromosome 12. Physical mapping was undertaken through the construction of a BAC library with a 9x approximate genome coverage. BAC clones from the apo-locus were obtained and used for FISH experiments confirming the hemizigosity and monosomic inheritance of the dominant marker as signals were obtained on a distal euchromatic region of one chromosome. Partial sequencing of BAC clones enabled the discovery of coding sequences at a low density and confirmed the synteny with rice chromosome 12. The functional characterization of candidate genes is underway via expression studies and mutant analysis in model plants.

10.1.6. Dissecting the control of diplosporous apomixis in *Tripsacum*, a wild relative of maize

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Apomixis refers to a group of highly variable developmental processes in angiosperms, leading to the formation of progenies that are genetically identical to their mother plants. Little is known about the molecular bases of apomixis. The current consensus is that apomixis results from the temporal or spatial alteration of the developmental pathways that in sexual plants lead to the development of the embryo, rather than the establishment of a novel function. We are using maize and its wild apomictic relative, *Tripsacum dactyloides*, to compare apomictic and sexual developments at the cellular and molecular levels in two closely related species. Our data indicate that both reproductive modes differ in the timing of expression of complete developmental pathways. We are using mutant analysis and specific genetic screens in maize to identify genes that regulate the temporal expression of genes during female reproduction and mimic aspects of the apomictic mode of reproduction. The results suggest that a surprising diversity of mutations can lead to apomictic-like features.

10.1.7. Genetic linkage mapping of an apomixis locus in *Taraxacum* (common dandelion; Asteraceae)

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In *Taraxacum*, the apomixis elements: diplospory (first division restitution meiosis), parthenogenesis, and autonomous endosperm formation, inherit independently, indicating control by different loci. Diplospory inherits as a dominant, monogenic trait: *DIPLOSPOROUS* (*DIP*). We mapped *DIP* by (1) bulked segregant analysis, followed by analysing 73 plants for 34 AFLP- and 2 SSR markers that co-segregate with diplospory, (2) screening of a total of 2229 plants for two markers close to, and spanning, *DIP*, followed by analysing the 24 recombinants found for the 6 AFLPs in this region, and (3) FISH experiments, using BACs that are positive for markers closest to *DIP*. The first map measured 18 cM, showing markers in groups at both sides of *DIP* at a minimum

distance of 1.4 cM. Recombination is not found in other mapping studies of apomeiosis to date, and allows for map-based cloning of *DIP* in *Taraxacum*. The second map showed the closest markers to *DIP* at a minimum distance of 0.1 cM and spanning 0.6 cM. First FISH results indicated co-hybridisation of BACs originating from both sides of *DIP*. This suggests a short physical length of the *DIP* chromosomal region, but has to be confirmed.

10.2.1. Insertion-tag mutations in *Arabidopsis* that affect tolerance to abiotic stresses

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Large-scale genetic screens were performed for stress response mutants using *Arabidopsis* harboring the luciferase reporter (*LUC*) driven by the stress-responsive *RD29A* promoter. These screens revealed lines whose functions describe essential or important components in stress signaling, and transcriptional and metabolic response networks. Selected mutants to be discussed include lines with differential stress responses to osmotic stress, low temperature, exogenous abscisic acid (ABA), or salt stress, or the ability to stress acclimate. Examples are mutants in the category (high expression of osmotically responsive genes). Mutant *hos10-1*, for example, a putative MYB transcription factor, impedes ABA biosynthesis. Additional mutants are those that enhance or repress known mutations in the SOS category (salt overly sensitive). Here, maintenance of ion homeostasis and oxidative stress protection are revealed, the latter connecting sodium-specific pathways to the injury response system.

10.2.2. Transcriptional changes associated with the abiotic stress tolerance of *Thellungiella*

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Thellungiella, a wild crucifer and close relative of *Arabidopsis*, is able to survive the extreme cold, dry and saline environment of the Canadian Yukon. We are investigating the molecular basis of *Thellungiella*'s stress tolerance using a cDNA microarray prepared from transcripts present in chamber-grown plants subjected to high salt, drought or cold conditions. The array of approximately 4000 different sequences was hybridized with probes prepared from plants subjected to each stress state with the objective to identify mRNAs that change in a "stress-specific" fashion. In addition we have harvested tissue from Yukon field sites to evaluate the transcript profile of plants experiencing multiple stresses. The results are being integrated with complementary physiological and metabolic studies. To date we have found surprisingly little overlap in the expression patterns amongst the three stress conditions.

10.2.3. Comparative genomics to identify genes for metal hyperaccumulation and hypertolerance in *Thlaspi caerulescens*

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Thlaspi caerulescens (*Tc*) is a natural Zn, Cd and Ni hyperaccumulator species belonging to the Brassicaceae family. It is a self-fertilizing species, closely related to *Arabidopsis thaliana* (*At*), with about 85-90% DNA identity in coding regions. While the physiology of metal accumulation in plants has received increasing attention in previous years, the molecular genetics is still largely unexplored. We address this by comparing gene expression in *Tc* and *At* exposed to deficient, sufficient and excess zinc concentrations. In addition we have sequenced a set of around 5000 ESTs from a root and a shoot *Tc* cDNA library. Besides several genes previously implicated in metal homeostasis, this comparative micro-array analysis revealed an interesting set of novel genes, which are differentially expressed either within each species or between them. The biological significance of the differentially expressed genes as well as interesting genes identified in the set of ESTs will be further discussed. This work is supported by an NWO-Genomics (050-10-166) grant, the EU-PHYTAC project QLRT-2001-00429 and the EU-RTN-METALHOME project HPRN-2002-00243

10.2.4. Transcriptional analysis of calcium-dependent and calcium-independent signaling pathways induced by oligogalacturonides

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-1,4-linked oligogalacturonides (OGs) are pectic fragments of plant cell walls, able to induce defense and developmental responses. To understand plant responses to OGs at the transcriptional level, we examined changes in gene expression using microarrays. In suspension-cultured *Arabidopsis thaliana* cells steady-state changes in the abundance of transcripts encoding stress- and disease-related proteins, signalling components, and transcription factors were particularly noteworthy. As in other plant cell types, OGs elicit a rapid - but transient - elevation in cytosolic free Ca²⁺. The Ca²⁺ transient can be abolished by various inhibitors, thereby facilitating distinction between Ca²⁺-dependent and Ca²⁺-independent transcriptional responses. Abolition of the Ca²⁺ transient specifically inhibited transcriptional upregulation of two groups: genes involved in post-pathogen attack cell wall modification and several genes involved in the biosynthesis of jasmonate (AOS and LOX) and of C6 volatile compounds (hydroperoxide lyase). Our data therefore suggest an essential role played by Ca²⁺ in the above processes.

10.2.5. Brassinosteroid mediated stress responses

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A remarkable feature of brassinosteroids (BRs) is their potential to increase stress tolerance in plants, but a good understanding of the molecular mechanism underlying this phenomenon is lacking. We have found that seedlings grown in the presence of 24-epibrassinolide (EBR) are more resistant to a variety of abiotic stresses than untreated seedlings. Investigation into the mechanism of EBR-mediated thermotolerance revealed that heat shock proteins accumulate to higher levels in EBR-treated seedlings, and this correlates with higher protein synthesis and modified translational machinery. Our present approaches to understanding how BR promotes stress tolerance include 1) overexpression of the DWF4 gene that mediates the putative rate-limiting step in BR biosynthesis, in *Brassica napus* and *Arabidopsis thaliana*, and analysis of the resulting transgenic plants, as well as of BR-deficient mutants for stress tolerance, and 2) identification of genes differentially expressed in BR-treated seedlings vs. untreated seedlings under non-stress and stress conditions by use of DNA microarrays. The results of these studies will be discussed.

10.2.6. Cold stress in white clover - An integrated view of metabolome and transcriptome responses

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Trifolium repens, with its capability to associate with symbiotic atmospheric N-fixating *Rhizobiaceae*, is the main forage legume in New Zealand pastures. Winter conditions lead to a decline of white clover in mixed pastures, the understanding of cold stress responses of clover is therefore of importance to New Zealand's agriculture. Flavonoids are known to be involved in general stress tolerance of plants and we studied the effects of cold stress on *T. repens* genotypes with low and high base levels of flavonoids. Changes in the transcriptome were analysed by microarray techniques using slides with clover specific cDNA's. Statistical analysis showed a strong cold induced regulation of genes involved in several biosynthetic pathways, including the flavonoid pathway. The metabolome was analysed by various analytical methods including GC-, and LC-MS/MS. Major metabolite classes differentially regulated by cold stress included flavonoids, sugars, sugar alcohols, and amino acids. We will discuss the importance of an integrated metabolome and transcriptome analysis for the unravelling of stress responses and future functional genomics strategies.

10.2.7. Linking physiological and genetic analyses with the modelling of drought tolerance

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While considerable progress has been achieved in improving plant survival to water stress by manipulating single genes, this has not been the case until now for improving the plant performances under water deficits compatible with agriculture. In this case, several mechanisms are involved, whose respective importances depend on climatic scenarios and whose genetic determinisms are still poorly understood. Thus, tolerance cannot yet be modelled directly from gene action. Another strategy is proposed, where the response of phenotypic variables to environmental conditions are considered *via* mechanisms at the plant level, resulting in models. Those can be tested by using transgenic plants affected on the mechanisms which drive the model. The parameters of models, usually parameters of response curves to environmental conditions, can then be analysed genetically and dissected into QTL effects. Genetic and ecophysiological models can be combined to predict the behaviour of virtual genotypes, as presented for maize leaf growth under water deficit. This opens the way to the simulation of virtual genotypes, known only by their alleles, in any climatic scenario.

10.3.1. Floral nectaries in angiosperms: a review of their distribution, evolution and systematic significance

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We present an overview of the distribution of floral nectaries in the major clades of the angiosperms using the APG II tree. It is argued that floral nectary types are systematically significant. Our earlier research on nectaries led to the identification of two main types: (1) persistent nectaries, grouping receptacular, gynoecial and gynopleural (or septal) nectaries, and (2) caducous nectaries, grouping phyllodial nectaries on the ephemeral perianth parts and androphylloinuous nectaries which are associated with stamens and staminodes. Gynopleural nectaries and caducous (staminal as well as tepal) nectaries characterise the monocotyledons. Disc-shaped gynoecial or receptacular nectaries never occur in monocotyledons. In eudicots, these disc-like nectaries are dominant. Gynopleural nectaries are excluded from "dicots" with some notable exceptions, which are the result of convergent evolution. It is striking that receptacular nectaries are the dominant type in rosids, while gynoecial nectaries are typical of asterids.

10.3.2. Nectar sugar composition in angiosperm families: phylogeny vs pollination

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The sugar composition of nectar in angiosperm flowers is traditionally interpreted as a functional character that relates mainly to pollination and pollinators. A systematic survey of thousands of nectar samples taken from many plant families has shown that the phylogeny of the plants is strongly reflected in the nectar sugar compositions. The relative quantities of fructose, glucose, sucrose and xylose are usually remarkably invariable within taxa, while they often differ markedly between taxa. Each plant family investigated had unique features and the sugar ratios varied from fairly uniform (Amaryllidaceae, Fabaceae, Orchidaceae), to moderately diverse (Acanthaceae, Crassulaceae, Ericaceae, Iridaceae, Proteaceae) to extremely diversified (Asphodelaceae, Geraniaceae). In some cases the nectar ratios are diagnostically different at generic and species level. The data support the idea that nectar sugars reflect the underlying genetic and phylogenetic relationships of the plants.

10.3.3. Nectary structure and nectar production in relation to flower symmetry and spur location in *Linaria genustifolia* (Scrophulariaceae)

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Various flower morphs of *Linaria genustifolia* were studied for their nectar production and floral-nectary structure. Most flowers are bilabiate and possess a single petal spur extending from the lower lip. The non-glandular spur holds nectar secreted by a crescent-shaped nectary that opposes the spur orifice. Nectary morphology of these 1-spurred zygomorphic flowers was compared with that of spurless zygomorphic and 3- to 5-spurred peloric, actinomorphic flowers. Mature nectaries in spurless flowers were slightly smaller. However, each peloric flower had a large, ring-shaped nectary

encircling the gynoecium. Stomatal distribution on mature nectaries also differed, being confined ventrally in zygomorphic flowers, but throughout the disk-shaped nectary in peloric flowers. Nectar production by the three flower morphs also differed. Nectar volume was highest in the multi-spurred flowers, intermediate in 1-spurred flowers, and usually nil in the spurless morph. Nectar-solute concentration, however, was reversed. On average, each peloric flower yielded about 3- and 400-fold more nectar sugar than the 1-spurred and spurless flowers, respectively.

10.3.4. On mechanisms of nectar secretion

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A comparative analysis of composition and ultrastructure including symplasmic connections of nectary vascular system and secretory cells was made in 15 species aimed at gaining an insight in mechanism of nectar transport. Plasmodesmatal frequency drastic reduction was found in symplasmic species. No intermediary cells characteristic of leaf minor veins of these species were differentiated in the nectaries. Thus, unlike leaves, the companion cells of nectary phloem in both apoplasmic and symplasmic plants play a passive role in assimilate transport. The significant proton gradient between apoplasm and symplasm of secretory cells was established in nectaries of three species using Sulphorhodamin G tracer procedure that indicate active secretion. Using gas chromatography the sucrose prevalence in nectar sugars was shown in two of these species and fructose - in one of them. Pathways of nectar discharge from the apoplasm to the nectary surface were the modified (always open) stomata, cuticular pores, and cuticular channels visualized by chemical fixation or by freeze substitution procedure. The apoplasmic mechanism of nectar secretion was proposed.

10.3.5. Secretory structures new to Anacardiaceae in two brazilian species

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Numerous secretory structures such as trichomes, idioblasts, ducts, extrafloral and floral nectaries have been reported in Anacardiaceae. The aim of this paper is to describe the glands of the vegetative and reproductive organs of *Spondias dulcis* and *Tapirira guianensis* from the anatomical and histochemical point of view. In both species, the shoot apex has colleters, idioblasts with heterogeneous secretion, and ducts associated with phloem and in the pith. The exudates of *T. guianensis* phloem and pith ducts are similar to each other, containing lipids and polysaccharides. In *S. dulcis*, the secretion is predominantly lipidic in the ducts associated with the phloem, and exclusively polysaccharidic in the pith. The flowers of *T. guianensis* have an intrastaminal ring and its epidermis produces lipids. This ring persists and remains secretory in the mature fruit where there is also a glandular endocarp. The two types of ducts producing different secretion compounds, colleters, idioblasts with heterogeneous secretion, intrastaminal ring producing lipophilic substances, and glandular endocarp have not previously been reported for Anacardiaceae.

10.3.6. Nectar carbohydrate sources: facts and hypothesis

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Nectar carbohydrates may be derived from photosynthesis directly or indirectly. In the former case photosynthesis may occur in the parenchyma of the nectary itself (photosynthetic parenchyma, PP) or in any other part of the flower or plant. There is little or no accumulation of starch in the PP. In the latter case the nectary has a storage parenchyma (SP) containing starch in the pre-secretory phase and the starch is hydrolysed in the secretion phase. SP is generally associated with large nectaries and a high rate of nectar secretion characterising short-living flowers visited by large pollinators. Photosynthetic parenchyma, on the other hand, is common in long-living flowers which secrete nectar over a long period and are visited by small pollinators. Defoliation experiments demonstrated that the direct contribution of nectary PP is not

sufficient to sustain the whole of nectar carbohydrate production. It derives that the plant must invest, in any case, part of the daily photosynthate in the production of nectar.

10.3.7. Nectar reabsorption and sugar translocation in male and female flowers of *Cucurbita pepo*

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The production and secretion of nectar has an energy cost that can be a substantial part of the energy economy of the plant. Plants may therefore recover part of the energy allocated to nectar secretion by reabsorbing nectar not collected by pollinators. This energy-saving strategy has been demonstrated by several authors by different methods. Here we demonstrate nectar reabsorption and sugar translocation in *Cucurbita pepo* by means of micro-autoradiography. Our results confirm that the dynamics of nectar reabsorption is different in male and female flowers. Differences in the dynamics of nectar reabsorption and sugar translocation were also found in pollinated and unpollinated female flowers. Pollinated female flowers reabsorbed sugar very quickly and translocated it to developing fruits in which ovules were the main sugar sink. Sugar translocation was slower and ovules did not label in unpollinated female flowers.

10.4.1. Dynamic genetics: experimental and computational approaches to the *Arabidopsis* shoot apical meristem

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The shoot apical meristem (SAM) is a permanent population of stem cells that provides for all of the above-ground tissue in a growing *Arabidopsis* plant. Despite consisting of only a few hundred cells, the SAM is highly structured, and planes, patterns, and numbers of cell divisions are highly regulated. The cells in the SAM control their division and patterns of gene expression based upon cell-cell communication. The SAM is a network of interacting cells that maintain their states and activities dynamically. We have developed a new set of methods for studying the SAM, and are developing computational methods for modeling it. The new analytic methods allow, via confocal microscopy and image analysis, three-dimensional time-lapse imaging over several days. Use of fluorescent markers allow visualization of gene expression domains and meristematic regions, and gene constructs of various sorts allow real-time manipulation of cell-cell communication. Computational modeling is in its early stages, but already sheds light on the possible mechanisms for primordium localization and meristem patterning.

10.4.2. Modelling genetic regulatory control of pea phenotype

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Ramosus mutants (*rms*) at five loci in garden pea (*Pisum sativum*) display increased branching in comparison to wild-type. The regulatory network of *RMS* genes and signalling processes must be deduced entirely through mutant-based grafting, and phenotypic, hormone and gene expression analyses.

As our biological understanding grows, there is an increasing need for computational modelling as a means to test whether hypotheses of the network are consistent with all data. Models are described as a list of hypotheses and then converted to a simple rule-based, algebraic formulation based on ideas from the mathematics of chemical processes.

We first compared a simple linear description of the process to a model involving feedback as predicted by grafting and hormone experiments and found that feedback was essential. This model led to the design of an unusual experiment to confirm some of its predictions. Testing against more recent *RMS1* gene expression studies resulted in the incorporation of novel ideas about subtle but important differences in the system in operation in the shoot and roots. Ongoing research is taking the developing plant structure into account.

10.4.3. A multiscale model of *Arabidopsis* development: from molecules to plant architecture

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A fascinating aim of current research is the understanding of the developmental processes through which multicellular organisms acquire their form. Mathematical models and computer simulations are an emerging methodology assisting in these studies. The talk will describe the work in progress aimed at creating an integrative model of *Arabidopsis thaliana*. The model takes into account the spiral phyllotaxis, apical dominance, flowering sequences, and architecture of wild type and selected mutant *Arabidopsis* plants. By combining computational models and simulations operating at different scales, we have begun to synthesize developmental processes from the level of molecules to the level of the whole plant architecture. This makes it possible to appreciate the link between the relatively simple mechanisms operating at the levels of plant modules and tissues and the resulting complex plant structures.

10.4.4. Computational modeling and image processing approaches to understanding the dynamics of the *Arabidopsis thaliana* shoot apical meristem

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An integrated effort to advance the understanding of the shoot apical meristem (SAM) of *Arabidopsis thaliana* through computational modeling of developmental processes, is embodied in the Computable Plant project. Interesting and generic mathematical problems arise at each stage of the computational approach. For example, quantifying the growth of the SAM and the lineages of its cells requires tracking multiple features in 3D imagery, which we approach through nonlinear optimization. Fitting the resulting data to a dynamical model requires a coupled mechanical/regulatory network modeling framework. For both the image processing and dynamic modeling we develop a mathematical foundation based on the use of a dynamical "grammar" capable of representing events such as cell division that change the number of objects present and their relationships, as well as continuous-time processes such as regulatory network dynamics and mechanical processes. The resulting computational algorithms are assisting experimental research on mechanisms of meristem maintenance and phyllotaxis.

10.4.5. Modeling traces 'crooked aspen' architecture to a single biomechanical factor

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A clump of trembling aspen (*Populus tremuloides*) located in western Canada exhibits an unusual 'crooked architecture', characterized by a shrubby short stature and sharply turning branches. It has been previously hypothesized that this architecture represents normal gravimorphic response to abnormal bending of current-year shoots. We use 3 models to explore this hypothesis. Model 1 reconstructs the bending of current-year shoots according to a time series of digitized 3D data. The model reveals that an axis bends near the base after growing some length. Model 2 simulates biomechanics of the development of a single axis. It shows that the observed shoot bending may result from the reduced stiffness of the axes, as suggested by anatomical evidence and biomechanical experiments. Model 3 simulates gravimorphic impact of shoot orientation on the development of the whole tree. The model demonstrates that the transition from the wild-type to the crooked morphology may be traced to a single factor, possibly a mutation in one gene, which reduces the stiffness of young shoots.

10.4.6. Towards a dynamic model of the *Arabidopsis* meristem

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During the last decade an impressive body of knowledge concerning shoot apical meristem function has been generated. This concerns information on the genes involved, their expression patterns, cell differentiation, cell division patterns, etc. The complexity of these data is such, that an integrated view of meristem function is not yet possible. Therefore, adapted mathematical and informatics approaches are now required to integrate the knowledge and to advance the level of understanding in the field. To formulate and test hypotheses on spatial aspects such as flows of signalling molecules between cells, strain within tissues, and the role of gene products in the spatial control of cell proliferation, we are creating a virtual meristem, that will integrate as much spatial, dynamic and quantitative information as possible. Here, we will present the first results obtained on the mathematical modelling of auxin fluxes in the meristem, based on experimental data. This modelling framework, based on local interaction hypotheses between cells, suggests that phyllotaxy patterns may emerge due to auxin overflowing in the meristem centre in growing meristems.

10.4.7. A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles

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The inputs for biologically realistic dynamic models of gene regulatory networks in cell differentiation are accumulating. A gene network model for floral organ cell specification grounded on experimental data shows that all initial conditions converge to few steady gene activity states that match expression profiles observed in primordial cells of sepals, petals, stamens and carpels and it also reproduces mutants patterns. Thus providing a dynamical explanation for the ABC model of floral organ determination, and showing that precise signaling pathways are not required to restrain cell types found in *Arabidopsis*, but these are rather determined by overall gene network structure and dynamics. This was confirmed by robustness analyses of random perturbations of gene interaction parameters. The network thus constitute a developmental module, and provides an explanation for overall conservation of floral plan among angiosperms. The model formally integrates available information, tests data coherence, predicts novel gene interactions, and enables explorations of evolutionary differences in network architecture between *Arabidopsis* and *Petunia*.

10.5.1. Land plant origins: body-building from scratch

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Land plants inherited their biochemistry from the green algae, but their morphology evolved on land. The foundations of the complex and integrated body plans that characterise the group were laid down during the ecological transition from water to land. This is borne out by recent work in molecular phylogenetics, the comparative morphology of the charophycean algae, and evidence from the fossil record. Early body fossils are microscopic, and interpreting this evidence is complicated by its invariably fragmentary nature and the difficulty of distinguishing sporophytic from gametophytic tissues. These findings have implications for our understanding of the origin of form, and they set limits on the extent to which fossils can usefully contribute to the debate on morphological evolution and phylogeny. The fossil record is silent with respect to the early stages of the development of multicellularity in plants, and it is likely to remain so. Despite this limitation, fossils are highly informative with respect to the assembly of other key elements of the plant body, capturing those durable aspects that appeared following terrestrialisation.

10.5.2. Tempo and mode of early land plant evolution: evidence from the dispersed spore fossil record

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The sporopollenin-walled spore was probably a prerequisite essential for the invasion of the land by plants, and is a character present in all subsequent land plants. Such spores have an excellent fossil record because they are produced in vast numbers, widely dispersed and have high fossilization potential. Thus the dispersed spore record is continuous and provides a unique insight into the timing of the origin of land plants and subsequent patterns of evolution, biodiversity and phylogeography. Intriguingly, following a first appearance in the Mid Ordovician, the spore record indicates a 40 million year period of apparent stasis. This is followed by a major diversification in the Late Silurian. These patterns may be interpreted as an extended period dominated by simple bryophyte-like plants, followed by an adaptive radiation in the Late Silurian coincident with the evolution of vascular plants. The pioneering bryophyte-like plants most likely were generalists with simple reproductive strategy. The later vascular plants had more complex ecologies and reproductive strategies, leading to ecological partitioning and phylogeographical differentiation.

10.5.3. Evolution of shoot development in land plants

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Diversity in form is a hallmark of extant species that was caused by the modifications of gene networks regulating development, and by the generation of novel developmental processes. The basic body plan of land plants, the shoot system, was established in their early stage of evolution. Homologues of the *SHOOTMERISTEM LESS (STM)* gene, which is a major regulator of shoot initiation and maintenance in flowering plants, were characterized in the fern *Ceratopteris richardii* and the moss *Physcomitrella patens*. Polar auxin transport was assessed in the moss. Basic functions of *STM* and polar auxin transport are preserved among their diploid generation, although the moss does not form shoot-like structure in its diploid generation. This suggests that basal genetic tools for shoot system were established in the shoot-less common ancestor of land plants. On the other hand, *STM* expression and polar auxin transport were not detected in the haploid leafy-shoots of the moss, suggesting that diploid shoot systems of the vascular plants and haploid shoot systems of the moss evolved with different molecular mechanisms and that diploid shoots evolved *de novo*.

10.5.4. The fossil record of leaf developmental and physiological evolution

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The fossil record indicates that the Paleozoic evolution of leaves among vascular plants consists of several independent acquisitions of laminate photosynthetic surfaces in different clades that previously had only axial structures. These independent evolutions of laminate leaves in lineages such as ferns, seed plants, sphenophylls, and archaeopterid progymnosperms follow a highly stereotyped sequence of morphologies. This pattern is hypothesized to result from constraints upon the convergent evolution of leaf development by modification of the mechanisms of axial development shared by all vascular plants. This hypothesis that convergent morphological evolution reflects convergent developmental evolution has been tested with developmental investigations of living tracheophytes. The detailed anatomical information available from plant fossils further allows comparative study of the evolution of hydraulic physiology in the leaves of different lineages.

10.5.5. Evolution of arbuscular mycorrhizal symbioses in early lineages of land plants

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Nearly all vascular plants have mutualistic arbuscular mycorrhizal symbioses where the plant host gains access to essential mineral nutrients through the fungus and the fungal symbiont acquires fixed carbon from the plant. However, the evolution of arbuscular mycorrhizal associations over the nearly 500 million-year course of

land plant history has been largely overlooked. Using environmental DNA amplification of 18S and ITS ribosomal DNA, we are the first to report on the identities of the arbuscular mycorrhizal fungal symbionts in a phylogenetically and ecologically diverse set of ferns and lycopods whose life cycles are characterized by a long-lived subterranean gametophyte phase. We have identified the fungal symbionts throughout the life cycle of these taxa including the autotrophic sporophyte and mycoheterotrophic gametophyte. Our data suggest that the evolution of mycoheterotrophic phases in ancient land plant lineages is highly homoplasious.

10.5.6. Do we need more characters or more taxa to resolve the overall pattern of land plant phylogeny?

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Despite theoretical cautions and accumulating empirical evidence to the contrary, there is a growing belief that the overall pattern of land plant phylogeny can be resolved using living species only. Great expectations and the impressive successes of molecular systematics in resolving relationships among relatively closely related species not withstanding, this approach has not led to confident resolution of the deep internal nodes of the tree. As a result, relationships among the four major clades of embryophytes, the pattern of phylogeny for living seed plants, and the overall pattern of phylogeny for embryophytes may be less confidently resolved today than they were a decade ago. To explore the possibility that alternate sets of characters, increased numbers of taxa, and well documented patterns of character development/evolution can provide reliable hypotheses tests, these approaches have been applied to questions about the deep internal nodes of the embryophyte tree. Preliminary results suggest that such approaches may serve as attractive alternatives to statistical assessments of hypothesis strength.

10.5.7. Fern shoot apical meristems (SAMs): structure, morphogenic activity and evolutionary homologies

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Investigation of SAM structure associated with organo- and histogenesis in five fern species differing in morphology and growth rhythm showed that: (1) the single apical cell (AC) and its immediate prismatic derivatives share common ultrastructural traits (high vacuolation, accumulation of starch and lipids) which characterize dormant seed plant SAMs; and (2) changes in ultrastructure associated with cell differentiation are correlated with successive unequal (formative) cell divisions of prismatic cells, accompanied by a decrease in plasmodesmata (PD) density. Although multicellular, fern SAMs are composed of clonally related cells, interconnected by primary PDs only (with frequency one order of magnitude higher than in seed plants), and both leaf and root ACs originate simultaneously from the SAM, not by cell proliferation, but with the emergence of the single AC. SAM structure correlates with shoot morphology. Leaf initiation in every prismatic segment of an AC, correlations between changing morphogenetic tempo, leaf shape, and stelar type are described for *Ceratopteris richardii*. Evolutionary homologies of the fern SAM are discussed.

10.6.1. Land plants and their fungi: co-evolutionary trends in symbiotic and parasitic interrelationships

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Mycotropism is a prerequisite for plants to conquer land habitats. Oldest symbiotic Glomeromycota are at least as old as first land plants. The arbuscular type of obligatory plant-fungus association has been established in all newly evolving land plants up to phanerogams. Surprisingly, Asco- and Basidiomycetes were involved in symbiotic interrelationships at a very early stage of plant and fungal evolution. In extant land plants, a distinct pattern of mycorrhizal associations (arbuscular, ericoid, arbutoid, orchid-type, ectomycorrhizae, ectendomycorrhizae) is present that indicates co-evolutionary traits. Only a minor percentage of land plants is considered to be non-mycorrhizal.

Plant life and development requires also decomposition of organic substances, both in living and dead material. There is only one chance for land plants' ecological and evolutionary success: a coordinated interrelationship with symbiotic, parasitic and saprobic organisms. A high diversity of plant parasitic and saprobic fungi is involved in these processes. In many cases, well defined associations occur that indicate co-evolutionary trends.

10.6.2. The role of fungi in shaping the early terrestrial ecosystem

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Fungi are critical in the world today. Recent reports place fungi in the Proterozoic. Some may represent fungi referable to modern groups, others examples of ancient lineages now extinct, and others modern contaminants or non fungal. The first fungal remains with structural and morphological features related to modern groups come from the ca 400 million year old Rhynie chert. Preservation provides information about fungi and host responses in this ecosystem. All fungal groups are present except basidiomycetes. Chytrids are common as parasites of algae, macroplants and other fungi. Features are identical to extant representatives, including life history biology. Endomycorrhizae are present in both life cycle phases of land plants, suggesting a strategy to distribute resources within the ecosystem. Endobacteria and a cyanolichen are also present. The Early Devonian is a critical period in the evolution of terrestrial ecosystems, when the first diverse communities appeared. The Rhynie chert provides the opportunity to document biodiversity in a fossil freshwater environment, and reveal community interactions in an ecological and evolutionary context.

10.6.3. Arbuscular mycorrhiza: an ancient symbiosis that can be cheated by plant parasites

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Arbuscular mycorrhiza (AM) is an extremely widespread symbiosis between the majority of plants and fungal symbionts from the phylum Glomeromycota. Fossil and molecular phylogenetic evidence suggests that this association has accompanied land plants since their origin. The main benefit provided to the plant by AM is an improved mineral uptake, which is essential under limiting conditions and less important in nutrient-rich soils. AM fungal diversity was shown to have a determining influence on the diversity and productivity of plant communities. Although host specificity in AM has generally been thought to be extremely low, studies using molecular identification methods have recently shown that in many cases there is at least some degree of host preference.

Some non-photosynthetic plants live in association with AM fungi and apparently derive all their nutrients via the fungal symbionts. These parasitic plants are known as mycoheterotrophs. Interestingly, they show a very high degree of host specificity, indicating that there is potential for specific interactions and co-evolution in AM, especially when the association is non-mutualistic.

10.6.4. How plant use fungal carbon: Evolution of orchids toward mycoheterotrophy

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Mycoheterotrophic (MH) plants are achlorophyllous and derive carbon from their associated fungi that are also mycorrhizal on autotrophic plants. MH plants are homoplastic among orchids: e.g. the tribe Neottieae encompasses green forest species and independently arisen MH species. As all MH orchids, MH neotroids shifted from rhizoctonias, the usual orchid mycorrhizal partners, to fungi ectomycorrhizal (ECM) on surrounding trees. To understand the predisposition of green neotroids to become MH, we studied *Limodorum*, *Epipactis* and *Cephalanthera* spp. for: (i) fungal symbionts, using molecular tools, (ii) carbon sources using stable C isotopes, and (iii) in situ gaz exchanges. These neotroids harbour no rhizoctonias, but ECM fungi, and use both fungal carbon and photosynthesis, i.e. are mixotrophic. Achlorophyllous

variants found in some green neotroids suggest that loss of photosynthesis may be a critical step in transition to MH states. Among Neottieae, ECM symbionts probably replaced rhizoctonias as neotroids ancestors colonized forest niches, allowing mixotrophy as an adaptation to low-light habitats. It predisposed neotroids to become MH.

10.6.5. Evolution of fungal endophytes of grasses, and the role of host specialization

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Grasses of subfamily Poöideae frequently host fungal endophytes of the family Clavicipitaceae, which systemically but asymptotically colonize aerial plant tissues and are transmitted efficiently to successive generations of host plants. These "epichloë endophytes" include the more pathogenic and obligately sexual *Epichloë* species, and mutualistic but asexual *Neotyphodium* species. Some *Epichloë* species are pleiotropic, i.e., also capable of mutualistic symbiosis. Phylogenetic evidence suggests that most *Epichloë* species and some *Neotyphodium* species have a history of codivergence with their hosts, dating from approximately 40 Myr before present. However, many *Neotyphodium* species are interspecific hybrids, whose ancestral *Epichloë* species are rarely associated with the same or related hosts. Some *Epichloë* species appear to have broad host ranges, but there is almost no detectable gene flow between populations on different host genera. The overall evolutionary pattern indicates host specialization as the overriding factor in speciation for all epichloë endophytes regardless of whether they are pathogenic, pleiotropic or mutualistic.

10.6.6. From local to global patterns of Caryophyllaceae-Microbotryum association: phylogenetic and population studies.

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What limits a fungal pathogen's host-range and geographic distribution is an urgent applied question, as well as informing upon the basic ecological processes that govern long-term species interactions. For obligate pathogens, such as the anther-smut fungus *Microbotryum*, their distribution is entirely constrained by that of the hosts and the ability to transmit between host species to which there may be varying levels of specialization. This study investigates the occurrence of *Microbotryum* on plant species in the family Caryophyllaceae found in Europe and North America. Phylogenetic analysis and inoculation studies indicate a high level of pathogen specialization to particular host species, but with evidence of historic and recent host-shifts between plant genera. Moreover, the phylo-geography of *Microbotryum* reveals the presence of the major European clade in northwestern North America, therefore suggesting a route for inter-continental migration of fungal pathogens in association with circum-polar hosts.

10.6.7. Comparing host and parasite phylogenies: Methods, principles, and an example from downy mildews

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Most, if not all, properties of host-parasite systems cannot be properly understood without referring to the respective phylogenies of hosts and parasites. The lecture shortly describes the current methods to reconstruct historical host-parasite associations including statistical tests of significant congruence between host and parasite trees. An important question here is how topological uncertainty related to parasite trees, host trees, or both is coped with. Most recent phylogenetic studies rely on molecular data; depending on the DNA (or protein) sequences the phylogenies are based on, alignment ambiguities may play a role, but may also be accounted for. These principles and methods are illustrated with phylogenetic data concerning *Hyaloperonospora* (downy mildews) and its plant hosts (Brassicaceae). The results are compared with conclusions drawn from other molecular phylogenetic studies in plant-parasitic fungi (mainly smuts) and their hosts as well as with literature data on host-parasite systems in other groups of

organisms. Finally, some general hypotheses on host-parasite evolution are proposed.

10.7.1. Phylogenetic relationships and patterns of diversification in Gesneriaceae subfamily Gesnerioideae

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Gesneriaceae subfamily Gesnerioideae includes more than 1800 species and is currently considered to include six tribes: Beslerieae, Episcieae, Gesnerieae, Gloxinieae, Napeantheae, and Sinningieae. We review the current understanding of phylogenetic relationships and morphological patterns in the subfamily based on combined molecular and morphological data sets. Combined nuclear and chloroplast sequence data and morphological cladistic data sets, suggest that in the Gloxinieae, circumscriptions of *Gloxinia* and *Phinaea* require reorganization. Similarly, in the Episcieae, several genera may require changes to generic circumscriptions, including *Alloplectus*, *Drymonia*, *Nematanthus*, and *Paradrymonia*, among others. Conversely, detailed studies of the Beslerieae suggest that generic definitions and phylogenetic clades are largely congruent. Morphological character variation will be compared with these phylogenetic clade definitions to assess potential morphological synapomorphies.

10.7.2. A molecular phylogenetic analysis of Coronantheroideae (Gesneriaceae).

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Coronantheroideae are unique among Gesneriaceae with nectaries adnate to ovaries, high chromosome counts, and a distribution bridging Old and New World tropics. The relationship of Coronantheroideae to other Gesneriaceae has been difficult due to autapomorphic traits that define it. In the present analysis four of six South Pacific/Australian and all three of the Southern South American genera are included. Analyses are based on four chloroplast loci, ITS and two nuclear low copy genes, glutamine synthetase and GCYC. Duplicate copies of both nuclear genes were encountered, probably a result of polyploidy. Phylogenetic analyses place Coronantheroideae with subfamily Gesnerioideae indicating that the ancestor to Coronantheroideae was from the New World tropics and its occurrence in Australia and the South Pacific is the result of two separate dispersal events. Estimates applied to the timing of dispersal allow resolution of whether the event was vicariant or long distance dispersal.

10.7.3. Phylogeny of Old World Gesneriaceae based on molecular and morphological markers

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The number of tribes recognised in Old World Gesneriaceae has steadily decreased, numbering presently four. Molecular studies continue this trend: only two major clades can be sharply delimited: (I) the small clade of Epithematoid Gesneriaceae, (II) the large clade of Didymocarpoideae Gesneriaceae. While in the former the relationships of the six genera analysed are fairly well understood, the Didymocarpoideae Gesneriaceae resist a neat grouping. Roughly, the following succession can be observed: (1) basal Asiatic and European genera, (2) African genera (primarily with twisted fruits), (3) advanced Asiatic genera, with (3a) genera with mainly twisted fruits, and (3b) genera with straight fruits. In this last group the intergeneric relationships are poorly resolved. This group includes also *Cyrtandra* with indehiscent fruits, while the second genus with indehiscent fruits, *Rhynchochotum*, is in the basal Asiatic group. The evolution of twisted fruits is either a parallelism or a single acquisition prior to the split between African and advanced Asian genera with a consecutive loss in the straight-fruited advanced Asiatic genera.

10.7.4. Cytology in Gesneriaceae after FISH

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The current state of cytology in Gesneriaceae is reviewed. While c. 1000 counts have been published, large gaps remain in our cytological knowledge as only 18% of the species have been analysed. There is some pattern in the distribution of basic chromosome numbers across the family. The New World Gesneriaceae show little intrageneric and intratribal variation, whereas the Coronantheroid gesneriads possess high somatic numbers. The Epithematoid gesneriads have low basic numbers and show high variation within some species. The Old World Didymocarpoideae gesneriads are cytologically the most diverse. The generally small size of Gesneriaceae chromosomes imposes limitations on genome characterisation. The application of fluorescent *in-situ* hybridisation (FISH) allows fine scale analyses into genome evolution. FISH can reveal differences in number and position of ribosomal DNA sites between closely related species with similar karyotypes (e.g. *Aeschynanthus*, *Streptocarpus*); indicating the power of this cytological tool for plants with small chromosomes.

10.7.5. Historical biogeography of Sinningieae and Nematanthus/Codonanthe lineages (Gesneriaceae): from regional to large scale patterns

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Distribution history of the tribe Sinningieae and the Nematanthus/Codonanthe group were inferred based on distribution data and molecular phylogenies using Dispersal-Vicariance analyses. Both lineages present their highest species concentration in the Brazilian Atlantic forests. However, biogeographical scenarios reconstructed in these groups are different. The Sinningieae probably arose in the Brazilian coastal rain forest. Dispersal events occurred predominantly into the neighboring inland areas, whereas few recent range expansions through the Andes account for the large peri-Amazonian distribution of the Sinningieae. By contrast, in the Nematanthus/Codonanthe, connections between the Brazilian Atlantic forests and the northern South America occurred early in the group, probably through the Amazon basin. These distinct biogeographic patterns correlate with differences in ecological traits. Our results stress the role of a phylogenetic framework to correctly infer biogeographic scenarios. This approach applied on an extended data set should contribute to better understand patterns of Gesneriaceae diversity in the Neotropics.

10.7.6. Systematics and biogeography of *Cyrtandra*, the largest genus of Gesneriaceae

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Taxonomic progress in large genera such as *Cyrtandra* (600 spp., Gesneriaceae) is hampered by an inadequate infrageneric classification. Preliminary phylogenetic analysis of ITS and trnL-F sequences for c. 100 species of *Cyrtandra* coupled with the identification of key morphological characters shows promise for building a taxonomic framework based upon monophyly. Using this structure, we can identify monophyletic groups of species or geographic areas containing monophyletic species radiations as targets for revisionary taxonomic work. In addition, the phylogeny has potential to address critical biogeographic questions in South East Asia such as the validity of biogeographic boundaries such as Wallace's line. Furthermore, the phylogenetic relatedness and age-structure of *Cyrtandra* species from particular regions gives insight into the evolution of tropical rain forest understorey communities.

10.7.7. Pollinator diversity of Gesneriaceae species from southeastern Brazil

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A notable diversity of species is harbored on Gesneriaceae, indicating adaptation to diversified pollinator agents. Pollinators

were inferred in many cases, but a few studies have determined the effective pollination system. Here we present field data of pollinator assemblages for 26 Gesneriaceae species. Pollination of these species is carried out by three animal groups: hummingbirds, bees, and bats. Hummingbirds belonging to Trochilinae or Phaethornithinae pollinate most species. Pollination by hummingbirds of each sub-family seems to be determined mostly by the plant habitat instead of floral traits. Four species are pollinated by small or large bees. Flower features (dimension, color, nectar) present marked differences which reflect their pollination by distinct groups of bees. Two species are pollinated by glossophagine bats, being nectar and scent attributes determinant for bat attraction. For most species pollination syndromes coincide with effective pollinators, but for a few species, pollinators belong to other groups than presumed by the syndrome. In the latter the actual pollination systems may be an endpoint or a transitional phase.

10.7.8. Fruit morphology and dispersal strategies in neotropical Gesneriaceae

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Fruit morphology is highly diverse in Gesneriaceae, especially in the neotropical tribes Episcieae, Gloxinieae and Sinningieae. Fruits are ovoid or globose, forming either dehiscent capsules (dry or fleshy) or indehiscent "berries". Fleshy and colourful fruits are common in Episcieae, but rare in Gloxinieae and Sinningieae. Habitat appears to play an important role in favouring appropriate seed dispersal mechanisms. Anemochory is more common in open and dry environments combined with lithophytic habit. Hydrochory is often found in ground-dwelling herbs in rain forest. Zoochory (supposedly bird-dispersed seeds) is mainly observed in epiphytic subshrubs and found predominantly in cloud or mountain forests. Some intrageneric variation also occurs, as illustrated by the genus *Sinningia*. In the Neotropics, Gesneriaceae have produced dispersal strategies that can be regarded as more innovative than in the Paleotropics, paralleling what happened with floral features and pollination mechanisms.

10.8.1. On the origin of the fig: phylogenetics and biogeography of Moraceae

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The majority of species in the mulberry family (Moraceae) are figs (*Ficus*), marked by a specialized inflorescence (syconium) and an obligate mutualism with pollinating fig wasps. Because of the unique morphology of the syconium, it has been difficult to investigate the evolutionary position of the fig. Phylogenetic analysis of chloroplast and nuclear DNA sequences have identified the closest relatives of the figs and suggest that the ancestor of the fig was insect-pollinated with protective bracts encircling the flowers. Molecular dating with multiple fossil calibrations, and independent geological evidence suggest that fig pollination is at least 80-90 million years old. Molecular evidence together with Eurasian fossils suggests that the early diversification of Moraceae in the northern hemisphere and subsequent dispersal into the southern hemisphere is at least as plausible as a southern hemisphere origin, if not more so. That independent dating of pollinating fig wasps favored a southern origin for fig pollination highlights the need for multiple sources of evidence in biogeographical reconstructions.

10.8.2. A new dated comprehensive phylogeny for *Ficus*: insight into classification and co-evolution.

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Figs (genus *Ficus*, family Moraceae) constitute one of the largest genera of flowering plants with ca. 750 species of trees, shrubs and hemi-epiphytes primarily occurring in rainforests world-wide. Figs can only be pollinated by female Agaonid wasps that can only oviposit inside the fig cavity. The extreme species-specific mutualism between figs and their pollinating wasps has become a model system for studies of co-evolution. However, an accurate evaluation of patterns and processes of species diversification in a co-evolution system can only be performed if the phylogenies of both partners are known and can therefore be compared. This lecture presents the most comprehensive phylogenetic analysis of

Ficus to date, based on nuclear encoded ITS and ETS sequences of about 150 species, showing both correlations and conflicts with traditional morphological classification. Independently inferred ages of closely associated fig and wasp lineages also provides the most compelling evidence to date for co-cladogenesis in the fig-wasp mutualism during the last 60 millions years.

10.8.3. Inflorescence evolution and phylogeny of Artocarpeae, an early diverging Moraceae lineage

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Artocarpeae is one of five tribes in the Moraceae (figs and mulberries) and is distributed throughout the tropics. The tribe includes the third largest genus in the family, *Artocarpus* (nearly 60 spp.), which comprises the pantropically cultivated breadfruit, *A. altilis*, and jackfruit, *A. heterophyllus* as well as several other species utilized for food or timber. The delimitation of the tribe and genera within it has been the subject of much uncertainty due to the difficulty in understanding the natural affinities of their complex inflorescences. Nuclear and plastid DNA sequences and morphological characters were used to reconstruct the phylogeny of Artocarpeae in order to test the monophyly of the current classification, trace the evolution of inflorescence characters, and identify the closest wild relatives of breadfruit and jackfruit. The results indicate that neither the tribe nor *Artocarpus* represent monophyletic lineages and provide a framework for guiding future classification and evolutionary studies of early diverging Moraceae lineages.

10.8.4. Phylogeny of *Castilleae* (Moraceae): Investigating the evolutionary history of the figs' closest relatives

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The tribe *Castilleae* (Moraceae) comprises 11 genera and 59 species distributed pantropically, including economically important species such as the Panama rubber tree (*Castilla elastica*). The sister lineage *Ficus* is well known for an obligate pollination mutualism with fig wasps. Despite the close relationship of *Castilleae* to these exemplars of coevolution, little is known about the evolutionary relationships or reproductive ecology within this group. DNA sequence data from the granule bound starch synthase gene and ITS as well as data from morphology were used in phylogenetic reconstruction. The phylogeny forms the foundation from which focused studies of character evolution, biogeography, and pollination biology are being conducted. This research represents the first phylogenetic investigation focused on species level relationships among *Castilleae*, and highlights the biology of an understudied plant group. The findings will contribute to understanding the origins of the fig-fig wasp mutualism, as well as the origins of plant diversity as 93% of *Castilleae* inhabit the Amazon basin, the most diverse tropical forest in the world.

10.8.5. Alternative utilization of food resource by a non-pollinating fig wasp and its implication on the fig - fig wasp mutualism

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We document here for the first time seed predation by kleptorasic fig wasps, which is used as a low quality resource to produce small males. In figs sampled during April 2003, from a tree of *Ficus citrifolia* we observed males of *Idarnes* sp. (*carne* group) developing within structures that resembled fig seeds and that differed for the galls from which they usually emerge. A comparative anatomical examination showed that the bored structures were in fact bored seeds. Males that had developed feeding on seeds were smaller in size than ones that fed on galls, suggesting that the quality of the food resource is better in galls. Our data points out that alternative utilization of good seeds as food resource occurs more intensely in figs with fewer galls available for oviposition. Bored seeds were also observed in other high-infested *F. citrifolia* crops in Brazil, suggesting that this alternative resource utilization may occur frequently. Alternative seed predation represents a direct cost to plant's female function, which makes this cleptorasic more effective parasites of the fig - fig wasp mutualism.

10.9.1. Rosid reproductive structures - progress and prospects

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With ca 70'000 species the rosids stand for more than a quarter of the total angiosperm species diversity. This taxonomic richness is reflected in a tremendous variety of floral organization and architecture. Rosids have in the recent past received extensive molecular phylogenetic study. As a result, monophyly and taxonomic composition of the group are well established. In addition, many subclades at the order level are now apparent. Deeper relationships, however, are still largely equivocal. As in many other parts of the plant tree of life, it will be impossible to reach an adequate understanding of the evolutionary history of the rosids without taking into account information from comparative morphological studies of extant and, in particular, also of fossil taxa. The fossil record of rosids is rich in well-preserved reproductive structures, and together with recent results from comparative studies of extant rosids, provides a wealth of structural data. Although much remains to be done at all levels, fresh attempts to synthesize and possibly reconcile results from molecular phylogenetics, comparative floral morphology, and palaeobotany, seem timely.

10.9.2. Fossil reproductive structures of Saxifragales and saxifrage evolution

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While much attention has been paid to utilization of fossils in calibration of evolutionary rates on phylogenetic trees, less has been devoted to exploring another application of fossils in a phylogenetic context: that of giving insight into character evolution within plant lineages. In this study, extinct taxa in the order Saxifragales represented by well-preserved fossils will be incorporated into a phylogenetic tree of extant plants (calculated using morphological and molecular sequence data) to explore the evolution of reproductive characters in the group over geologic time. By incorporating fossils into analyses with extant taxa and examining the optimization of morphological characters on the resultant tree(s), it is possible to explore the order in which particular traits first appeared and to discover unique, derived characters (or character combinations) in extinct lineages of plants, phenomena that cannot necessarily be discovered through examination of extant lineages alone.

10.9.3. Reproductive structures of Leguminosae: phylogenetic and paleobotanical utility

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The Leguminosae is the largest family in the rosid clade and its reproductive structures exhibit a great deal of morphological diversity. The fossil record of legumes is extensive and extends to the late Paleocene, with considerable taxonomic diversity appearing in the early to middle Eocene. Compression fossils of fruits and leaves are abundant and many have distinctive morphologies that are taxonomically diagnostic, but fossil flowers are relatively rare and often difficult to identify. Many other fossils represent dispersed organs that by themselves are not distinctive and therefore cannot be identified with precision. As a consequence, most legume clades that are known from the fossil record are documented based on fruits or leaves, but they are largely taxa that have distinctive morphologies. Ongoing phylogenetic studies of caesalpinoid legumes using molecular and morphological data will provide a robust phylogeny that, when fossils are incorporated, can be used to assess patterns and timing of morphological evolution in the diversification of this important family.

10.9.4. Evolutionary idiosyncrasies of floral features in rosids

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The new systematic circumscription of orders and higher groups of rosids based on molecular phylogenetic studies has revealed blatant lacunae in our knowledge on structural and biological characteristics of the new groups. New floral studies that go

beyond traditionally considered features in larger clades of rosids may provide insight, not only into potential synapomorphies for these groups, but they are also beginning to give fresh insight into the evolutionary stability and lability of features. Such insight allows for the evolutionary behaviour and correlation of certain features at the level of the rosids or subclades of rosids. Although such studies are both time-consuming and difficult, beginning with the availability of liquid-fixed flowers at comparative stages of development, they are greatly needed for progress in evolutionary research. Better knowledge of the idiosyncrasies of flowers of extant plants will also be helpful for the systematic interpretation of fossil flowers.

10.9.5. Flowers and phylogenetic affinities of Normapolles plants - a complex of extinct fagalean lineages

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The Normapolles complex, defined by its characteristic triporate pollen, constitutes an important and diverse element of many Late Cretaceous and Early Cainozoic floras of the Northern Hemisphere. Based on the dispersed pollen record alone it has been difficult to assess systematic affinities, but relationships with Fagales had been proposed. Over the past twenty years several, exquisitely preserved, Late Cretaceous inflorescences and flowers with Normapolles type pollen *in situ* have been described. On the basis of their general inflorescence structure, floral organisation, and pollen features they all have been suggested to be related to various members of Fagales, in particular to members of the clade comprising Betulaceae, Casuarinaceae, Juglandaceae, Myricaceae, Rhoipteleaceae, and Ticodendraceae. We present the results of a re-examination and synthesis of floral organisation and structure in Cretaceous Normapolles flowers and test the supposed paraphyly of the Normapolles complex by means of a phylogenetic analysis.

10.9.6. Floral structure and systematics in larger clades of rosids

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Phylogenetic studies have greatly impacted upon the circumscription of taxa within the rosid clade, resulting in novel relationships at all systematic levels. In many cases, the floral structure of these taxa has never been compared, and in some families, even studies of their floral structure are lacking. Our aim has therefore been to concentrate on poorly studied and basal taxa within new orders, and within orders whose circumscription has significantly changed from traditional classifications. Specifically, to compile detailed analyses of floral structure for these taxa, and to make comparisons at both the intraordinal and interordinal levels, with the aim of deriving potential synapomorphies for these groups. We have focussed upon shared features that are less common among angiosperms. Lack of phylogenetic resolution however, at the base of rosids, does not (yet) allow for further interpretation of these features. Thus far, we have investigated the orders Crossosomatales, Cucurbitales, Oxalidales and Celastrales and will present overviews of these studies, including discussion of potential synapomorphies for each of these groups.

10.9.7. Flower evolution in Malvales with emphasis on the androecium

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The order Malvales comprises Malvaceae s.l. (including former Tiliaceae, Sterculiaceae, and Bombacaceae) and ten smaller, less-known families (Bixaceae, Cistaceae, Cochlospermaceae, Diegodendraceae, Dipterocarpaceae, Muntingiaceae, Neuradaceae, Sarcolaenaceae, Sphaerosepalaceae, and

Thymelaeaceae). Molecular data support several subclades within the order, but deeper nodes remain largely unresolved. Neuradaceae, many Thymelaeaceae, some Dipterocarpaceae, some Malvaceae, and a few Sarcolaenaceae are characterized by a low number of stamens, which are arranged in one or two whorls. We show that the androecia of most other taxa of Malvales have numerous stamens, which often develop on a complex ring primordium in a centrifugal or more complicated pattern. Nonetheless, the androecia of these families have apparently a basically two-whorled ground plan. Furthermore, even the highly modified androecia of Malvaceae with androecial tubes and androecial lobes upon which 'half-anthers' are inserted seem to exhibit developmental and vascular patterns suggestive of a two-whorled organization.

10.10.1. Molecular systematic, floral developmental and anatomical revelations on generic relationships and evolutionary patterns in the Violaceae

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Recently completed molecular systematic investigations of the Violaceae using the chloroplast trnL-F spacer confirm prior rbcL results, rejecting subfamily Leonioideae and all traditional tribal groupings as non-monophyletic. The genus *Hybanthus*, defined by an enlarged saccate petal, is highly polyphyletic, comprising six divergent and biogeographically coherent lineages dispersed across the family. Floral developmental studies reveal distinctly zygomorphic corollas in mid-development of all studied genera, with "actinomorphy" achieved late in ontogeny; the family may have a zygomorphic, rather than actinomorphic, recent common ancestor. Developmental results clarify filament fusion and nectary morphology across genera. Xylem anatomy, palynology, chromosome numbers, calcium oxalate crystal morphology and distribution, androecial and other morphological traits, and biogeography support the numerous novel clades and generic placements from the chloroplast phylogenies. Suites of some of these traits provide the means to circumscribe and distinguish the six generic segregates currently lurking under *Hybanthus*.

10.10.2. Dismantling the polyphyletic genus *Hybanthus* Jacq. (Violaceae)

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The genus *Hybanthus* Jacq. differs from other Violaceae by its enlarged bottom petal and saccate base. Revisionary studies suggest over 100 species in the genus. Molecular phylogenetic analyses of the family using rbcL and trnL-F spacer sequences reveal *Hybanthus* as highly polyphyletic. Further investigation of the seven biogeographically coherent chloroplast clades has uncovered 12 morphological, anatomical and cytogenetic features circumscribing the clades, including corolla morphology, androecial morphology, fruit and seed morphology, calcium oxalate crystal morphology and distribution, and base chromosome number, with suggestions of further traits from embryos, pollen and vessel elements. Genera to segregate from *Hybanthus* sensu lato are (1) *Hybanthus* s. str. in Mesoamerica and the Caribbean [6 spp.]; (2) *Cubelium* Raf. in eastern North America [1 sp.]; (3 and 4) two unnamed groups in Mesoamerica [2 spp. each]; (5) *Pombalia* Vand. in the southwestern US and Latin America [55 spp.]; (6) *Pigea* DC. in South Africa, southern Australia and New Caledonia [15 spp.]; and (7) an unnamed group in Africa, Indochina and Southeast Asia [15-25 spp.].

10.10.3. Studies on the new endemic genus *Hybanthopsis* and other Brazilian hybanthoid violets

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The tribe Violeae is represented in Brazil by ca. 40 spp., most of them belonging to *Hybanthus* (27 spp.), and the remaining is distributed among *Anchietea*, *Corynostylis*, *Noisettia*, *Schweiggeria* and *Viola*. Recently, the tribe was increased by another genus, with the description of *Hybanthopsis*, with a single species, *H. bahiensis* Paula-Souza, endemic to "caatingas" (xerophytic, deciduous vegetation) in Eastern Brazil. This is the fourth lianescent genus of the family, along with the Neotropical

Anchietea and *Corynostylis*, and the South Pacific *Agatea* (also included at the Violeae). The phylogenetic relationships of the new genus are still unclear, but it is expected that it falls within the other 3 lianescent genera, which are grouped into a well-supported clade on preliminary studies on the phylogeny of the family. Although the flower structure of *Hybanthopsis* is most similar to *Hybanthus* or the lianescent *Agatea*, it is probably more closely allied to *Anchietea* due to its chartaceous capsule and semi-obsolete ridge on the seeds. The new genus is just one of the several novelties within the tribe Violeae on recent taxonomic studies with the family in Brazil.

10.10.4. Revision of African *Rinorea*: Diagnostic characters

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Botanists working on African *Rinorea* have all experienced the difficulties of distinguishing species. The question was well highlighted by De Wildeman (1920), who published a revision of this group, building on the work of Engler (1904) and Brandt (1914). He stressed the need to discriminate *Rinorea* by using many stable, criteria, avoiding the use of variable characters. However, his resultant tentative keys remain unsatisfactory and their use has led to confusion in *Rinorea* identifications.

To avoid these shortcomings, it is proposed here to base *Rinorea* delimitation on the correlation between primary and secondary characters as suggested by Leonard (1955). Primary characters are those located in flowers and particularly in the androecium; secondary characters are those shown by other structures. These correlations allow an improved delimitation of sections in the genus *Rinorea*. Within the sections, it then becomes easier to discriminate species. The aim of this paper is to suggest guidance on dividing African *Rinorea* into sections, and sections into species.

10.10.5. Lilliputian wonders in the Rosulate Violas (sect. *Andinium*) of Southern South America

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Approximately 100 species of dwarf annual and perennial herbs comprise section *Andinium* of *Viola*, the so-called Andean rosulates. They range fairly continuously over 5400 km of longitude between the equator and Santa Cruz province, Argentina, and occur within five Andean nations. Although mainly alpine in vertical distribution and ecology, a few also descend to the Pacific coast. Of rather variable habit, their typical and most notable form consists of compressed or columnar rosettes a few cm high, these more like certain Crassulaceae or saxifragas than other known violets. Foliage is often arranged tightly in geometrical spirals. Flowers however are conservative and instantly betray their genus. Rosulates are frequent pioneers, associated with volcanic orogeny and sites of little or no other cover. Many seem unable to tolerate direct competition. Other tendencies include mimetic coloration, spot or narrow endemism and small populations. *Andinium* contains a quarter of all *Viola* species, yet is hardly known. The taxonomy is undeveloped, having scarcely been studied for 75 years and never in its entirety. That situation is currently being addressed.

10.10.6. Allopolyploid evolution of the temperate Violas - results from chloroplast and nuclear gene markers

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Data from morphology, biogeography, chromosome numbers, crossing compatibilities and DNA sequence variation (ITS1&2) have been used to construct phylogenies of *Viola*. Together, they suggest that (i) the genus originated in South America, that (ii) its base chromosome number is $2n=12$ and that (iii) the blue-flowered temperate groups, having as base numbers $2n=20$ (section *Viola*) and $2n=24$ (section *Plagiostigma*), originated independently from within the basal, yellow-flowered section *Chamaemelanium* ($2n=12$). While all investigated *Viola* groups possess only a single ITS sequence type, isozyme studies have shown that most groups having $2n=20$ or higher numbers are in fact polyploid. The hybrid origins of these groups have so far not been fully resolved. We have examined polyploid groups of *Viola* using chloroplast DNA and low-copy nuclear gene markers. In contrast to ITS sequences, paralogues of low-copy nuclear genes are conserved in the

polyploid groups. Chloroplasts are non-recombining and suitable to retrieve maternal lineages. Our preliminary results show that these markers are promising tools in disentangling the polyploid evolution within *Viola*.

10.10.7. Population features of hybridization in *Viola*: two case studies

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Hybridization is a widespread phenomenon in *Viola*. Its extent and impacts in natural populations are documented: Allozyme analysis of five species of the sect. *Viola* and their hybrids along three transects show that hybrids are very common in natural populations, hybridization frequently operates across the ploidy levels, hybrids with one or both parents paleoecoploid are sterile. Most of the seed production of fertile hybrids comes from kleistogamous flowers, and chasmogamous flowers have much lower fertility, spatial distribution of hybrids along the transects reflects the ecological preferences of parents. Functionality of chasmogamous flowers in hybridization and the massive cleistogamy suggest a complex population structure in the sect. *Viola*. Introgression between a native, subendemic *V. lutea* subsp. *sudetica* ($2n=50$) and an introduced species, *V. tricolor* s. lat. ($2n=26$) was studied. Karyology shows a rapid spreading and persistence of the hybrid plants, formation of hybrid types having chromosome features outside the ranges of parents, and strong introgressive influence on the native parent about 25 years after the initial hybrid contact.

10.11.1. Land use history and forest plant metapopulations in dynamic landscapes

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In fragmented landscapes, habitat patches are often destroyed and created through time, though most metapopulation models treat patch networks as static. Numerical simulations of classic Levins-type models can be used to demonstrate that for long-lived plants, such as forest herbs, historical land use can create disequilibria between landscape structure and patch occupancy that may endure for decades to centuries. We have also developed a generally-applicable, modified version of Hanski's Incidence Function Model (IFM) that incorporates landscape dynamics (i.e., habitat patch turnover), and we have parameterized the model with data on patch occupancy patterns for forest plants in central Lincolnshire, UK. Estimated colonization and extinction rates varied significantly among species with different life-history traits. Model simulations demonstrate a profound negative influence of habitat turnover rate on metapopulation dynamics and persistence, particularly for slow-colonizing species.

10.11.2. Genetic diversity and metapopulation dynamics in Mediterranean endemics

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Plant species often have a patchy and unstable distribution. When metapopulation dynamics is such that recolonization rate is larger than extinction rate, the species is actually healthy from a demographic point of view. Some species, conversely, appear as collections of highly stable local populations with little exchange among them, so that their viability is entirely dependent upon each population viability. When few populations constitute such species, they are highly threatened. I will describe the demographic and genetic work we are conducting on two endemic plant species living in rocky habitats of the Mediterranean basin (*Centaurea corymbosa* Pourret and *Brassica insularis* L.). Because they are characterized by highly stable local populations, they might not appear as highly threatened as other endemic species living in more human-disturbed environments. However these species are also characterized by small population sizes, so that demographic, genetic and environmental stochasticities might actually constitute major threats for their viability.

10.11.3. Effects of inter-population crosses between fragmented populations of the common *Lychnis flos-cuculi*

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To evaluate the consequence of adaptation to landscape heterogeneity and fragmentation, we use the common but declining *Lychnis flos-cuculi*. We know that our population show local adaptation to environmental gradient and plants fitness components present an allee effects when comparing large and small populations. In spring 2003 we crossed 7 plants of each of 13 populations with a plant from: 1/ the same population, 2/ a small, 3/ a large, 4/ an ecologically similar and 5/ an ecologically dissimilar population. Crosses between populations yielded 26 % more seeds per fruit ($p<0.001$), which weighed 7 % less ($p<0.05$), indicating hybrid vigour for early fitness of the first generation of outcrossing. In the field, larger population produced higher flowering stems ($p<0.05$) but only when crossed with other large populations, indicating a detrimental effect of outbreeding for large populations. Currently we are growing some offspring in the greenhouse, other in the parental populations, and we produced F2 and backcrosses. Our results will allow to balance positive effects of heterosis and negative effects of disruption of adaptation in outbreeding crosses.

10.11.4. Predicting the fate of species in fragmented landscape based on their demography and dispersal ability

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Effect of habitat fragmentation on species survival is a major concern of species conservation. While there are a few model studies predicting the effect of landscape changes on survival of single species, the possibility to generalize these conclusions is still largely lacking. The aim of this study is thus to make general predictions on how species respond to future landscape change depending on their traits. Specifically, we want to examine how local population dynamics and dispersal affect prospect of species' survival at a landscape level. We used a spatially explicit metapopulation model and calibrated it with real data of a range of plant species. Data on local population dynamics were extracted from literature; data on wind dispersal distances were simulated using the model of Tackenberg (2003). To evaluate response of the species to landscape changes, we assessed survival of the species, their population size and habitat occupancy under different scenarios of habitat removal and frequency of local disturbance. The results show how species survival depends on local population dynamics as well as on its dispersal ability.

10.11.5. Metapopulation biology of a declining, poorly dispersed plant species in Brandenburg: a project towards efficient habitat networks in plant conservation

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While systems of connected plant habitats have become legal requirements for plant conservation, guidelines for their design are scarce and their actual success is largely unknown. According to metapopulation theory species persist if local extinctions are balanced by (re)colonisations. While most studies in plant conservation biology concentrate on population viability and neglect colonisation, we study ecological and genetic determinants of both for the still common *Armeria elongata* in its patchily distributed habitats in the German federal state of Brandenburg. There, the viability of its populations is threatened by agricultural change, and the colonisation of new habitats is hampered by its poor seed dispersal. With spatially-explicit observations and experiments we address the dynamics of populations and metapopulations and their environmental and genetic drivers. We selected 24 extant populations for detailed demographic and genetic monitoring, and further monitor suitable, but currently unoccupied, sites. Our comprehensive approach aims to help adapting habitat networks to actual requirements of plant conservation.

10.11.6. Lattice models of plant populations with spatial local interaction between individuals

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Immobility of plants requires special mathematical methods for simulation of population dynamics. Simplification of the models can be done using lattice presentation of the population space. In this case cellular automata approach to simulation of plant populations or community dynamics can be developed and computer experiments can be carried out. Uniting this modeling approach with the concept of discrete description of plant ontogeny allows for developing of a set of discrete models for analyzing the spatial-temporal dynamics of plant populations and communities. Local interactions between species and individuals in terms of neighborhood can be introduced allowing for description of nonlinear population dynamics. Short-rhizome plants' population nonlinear dynamics at random extinction of individuals with different intensities, the role of asynchronous changes of age states, the rise of stable spatial structures from initial random pattern were studied. Events similar to phase transitions, conditions of population stability were found for different plant's life forms.

10.11.7. A comparison of life-history characters of arctic and alpine populations of the annual *Koenigia islandica*.

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Major differences in environmental characteristics between arctic and alpine ecosystems exist, to which plants having populations in both arctic and alpine environments must adapt differently.

Population differentiation in life-history characters was tested for several populations of *Koenigia islandica*, an arctic-alpine annual with a wide, circumpolar distribution, by germinating and growing each population under simulated arctic and alpine conditions.

Several life-history characters are predicted to differ between arctic and alpine populations, including seed germination and reproductive strategies, as well as vegetative growth. For example, plants from northern populations grew better under simulated arctic conditions, reaching larger sizes and producing more flowers and seeds. Plants originating from low latitude environments seemed unable to benefit from the long photoperiods of arctic conditions, developing poorly and reaching smaller sizes. Also, seed stratification had different effects on the germinability of different populations.

10.12.1. Molecular, biochemical and phenotypic definition of the physiological impact of V-PPases on plants

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Vacuolar pyrophosphate- (PPI-) energized proton pumps (V-PPases) were originally thought to be restricted to plants and comprise a category of structurally and functionally uniform membrane proteins. Recent studies, however, have established that these primary pumps are not only ancient, widespread and pertinent to the metabolism of many organisms including archaeobacteria, proteobacteria and parasitic protists but also fall into two distinct subcategories, termed type I and type II. To define the impact these novel energy transducers have on the intact organism we have, using *Arabidopsis thaliana* as a model plant system, succeeded in isolating T-DNA insertion mutants for all of the key genes. Specifically, we now have three types of null mutant for the sole gene encoding the type I V-PPase and both of the genes encoding the type II V-PPases, and have assembled a broad range of probes for distinguishing type I and type II V-PPases at the molecular, protein and functional levels. The properties of these mutants, and what is known and what remains to be determined of the fundamental biochemical capabilities of V-PPases will be discussed.

10.12.2. The H⁺-PPase AVP1 is required for organ development in *Arabidopsis*

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Cells expend as much as 50% of their total intracellular energy reserves to maintain gradients of ions across their membranes. The electrochemical potential of these ion gradients represents stored energy. Plants and fungi are similar in that they use proton (H⁺) gradients as the "currency" with which to mediate transport of ions, whereas animal cells use Na⁺ gradients as the driving force. The H⁺-PPase AVP1 is classically thought of as maintaining the acidic nature of the plant vacuolar compartment. Here we report that AVP1 also plays a critical role in facilitating auxin transport and thereby coordinating development. AVP1 overexpression resulted in enhanced cell divisions at the onset of organ formation, hyperplasia, and increased auxin transport. In contrast, avp1-1 null mutants have severely disrupted root and shoot development and reduced auxin transport. AVP1 was localized to both the tonoplast and plasma membrane where it likely contributes to H⁺-mediated auxin influx. We conclude that AVP1 plays an important role in organ development through facilitating the auxin fluxes that regulate organogenesis.

10.12.3. The role of inorganic pyrophosphate as an alternative energy donor at low oxygen

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In contrast to animals, plants lack specialised circulation systems, and oxygen falls to low levels within many plant tissues. A specific role of PPI in conserving oxygen will be discussed. The cytosol of the plant cell contains significant levels of PPI, which are maintained at high levels in hypoxic tissues. PPI is utilised as an energy donor for sucrose mobilisation via sucrose synthase and UDPglucose pyrophosphorylase, for glycolysis via PPI-dependent PFK, and for tonoplast energisation via a PPI-dependent proton pump, each of these enzymes being induced by low oxygen. Each PPI dependent reaction actually duplicates an ATP consuming reaction, which in the case of invertase and the ATP-dependent proton pump are repressed under low oxygen. When sucrose synthase is bypassed by a route that utilises ATP instead of PPI, tissue oxygen tension and cellular energy state decrease. This provides evidence for an important role of PPI in recycling waste energy to fuel important central metabolic and cellular functions, thereby allowing both ATP and oxygen consumption to be decreased.

10.12.4. An ancient pyrophosphate-based stress bioenergetics was preserved during the evolution of the photosynthetic lineage

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Inorganic pyrophosphate (PPI) could arise through a variety of abiogenic processes in the primitive Earth. It is conceivable therefore that organisms in the earlier stages of biological evolution developed a PPI-based bioenergetics. Recent evidences indicate that proton-pumping pyrophosphatase (H⁺-PPase), a key protein of that "primitive" energy metabolism, is present in a wide range of extant organisms of the three domains of life, although cyanobacteria, fungi and metazoa are remarkable exceptions. A prevalent feature of organisms having H⁺-PPases is that they must chronically cope with a variety of environmental conditions -many of them resembling those of primitive Earth- that severely constrain the cell energy status (anoxia, extreme physico-chemical conditions, nutrient scarcity, etc.). We have found specific regulations at the genetic level of *R. rubrum* and algal H⁺-PPases that support this proposal. An outstanding paradigm of this lifestyle is the photosynthetic lineage, from prokaryotes to plants, that includes the only multicellular organisms (metaphyta) in which H⁺-PPases have been found so far (*supported by MEC BMC2001-563 grant*).

10.12.5. The H⁺-pyrophosphatase is present in both prokaryotic and eukaryotic acidocalcisomes

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The H⁺-pyrophosphatase has been shown to be a useful marker for acidocalcisomes. Acidocalcisomes are dense, acidic organelles with a high concentration of phosphorus present as pyrophosphate and polyphosphate complexed with calcium, and other cations. The acidocalcisome membrane contains a number of pumps

(Ca²⁺-ATPase, V-H⁺-ATPase, H⁺-PPase), exchangers (Na⁺/H⁺, Ca²⁺/H⁺), and channels (aquaporins), while its matrix contains enzymes related to pyrophosphate and polyphosphate metabolism (exopolyphosphatase, polyphosphate kinase, pyrophosphatase). Acidocalcisomes have been found in several pathogenic microorganisms as well as in the green alga *Chlamydomonas reinhardtii*, and the slime mould *Dictyostelium discoideum*. The identification of acidocalcisomes in bacteria and the finding that human platelet dense granules are homologous to acidocalcisomes, indicate that these organelles have been conserved from bacteria to humans. Acidocalcisomes function in the storage of cations and phosphorus, pyrophosphate and polyphosphate metabolism, calcium homeostasis, maintenance of intracellular pH homeostasis, and osmoregulation.

10.13.1. Engineering rubisco to improve yield: future potential or sisyphian task.

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Most projections paint a sobering picture of the ability of agriculture to keep delivering the yield increases necessary to support the future global population. By 2050, if there is only marginal improvement in field performance of major crops there could be a serious shortfall in production. Researchers are working intensively to find ways of sustaining the impressive increase in productivity of past years, but this might be impossible without turning over more land to agriculture; an unlikely scenario. Many of the strategies will necessarily involve engineering plants, but it takes many years to go from the lab to the field with a new stable variety that shows robust yield improvement. Furthermore, identifying which trait would provide a significant boost to yield is becoming a major challenge.

It is well recognized that a major constraint to plant productivity resides at the first step in C-fixation and alleviating this limitation could have a significant impact on yield. Although many of the necessary technological components to achieve this goal have been developed, putting them together will be a long-term undertaking.

10.13.2. Make or Break: Metabolism of the rubisco inhibitor, CA1P

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We have recently established a route for the *de novo* biosynthesis of the naturally occurring Rubisco inhibitor, 2-carboxy-D-arabinitol 1-phosphate (CA1P), from recently assimilated carbon in the Calvin cycle. CA1P is present in many plant species at night and is broken down in the day by a specific phosphatase. We are currently attempting to identify and clone the genes involved in the biosynthesis and metabolism of CA1P, including those for the widely distributed but little studied branch chain sugar, D-hamamelose (2-hydroxymethyl-D-ribose) and its phosphate esters, which are intermediates in the biosynthesis of CA1P. In the course of this work, a gene thought to encode a CA1P-specific phosphatase has been cloned and Arabidopsis knock-out mutants obtained. This gene has been shown to direct the synthesis of a protein with CA1P phosphatase activity when incorporated into an *in vitro* wheat expression system. Oligonucleotide microarrays have also been used to examine hamamelose-induced differential gene expression in leaves of Arabidopsis.

10.13.3. Structural framework for catalysis and specificity in rubisco

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Photosynthetic efficiency is limited by the catalytic properties of the major photosynthetic CO₂-fixing enzyme, Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase). Three-dimensional structures for Rubisco have been determined from several prokaryotic and eukaryotic sources. While these structures have provided a wealth of information and present an important framework for structure-function studies, no simple rationale for engineering a better enzyme has presented itself so far. However, results from genetic screening and site-directed mutagenesis show that amino acid substitutions in several distinct areas of the

enzyme influence the catalytic properties of Rubisco. A survey of key structural features of both natural and engineered Rubisco variants will be presented along with results from recent studies on the molecular basis for the catalytic diversity of Rubisco.

10.13.4. Altering photosynthetic carbon fixation through rubisco engineering

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Over the last decade, efforts to manipulate the photosynthetic CO₂-fixing enzyme, Rubisco, *in planta* by plastome-transformation technology have advanced considerably. Rubisco's catalytic properties have a profound impact on the efficiency of the photosynthetic process making it an obvious target for manipulation. The surgical precision of the homologous-recombination mechanism that enables plastome manipulation provides an excellent tool for testing our knowledge, and our predictions, about Rubisco biology. My groups ongoing plastome-transformation projects seek to address different aspects of Rubisco biology - its catalytic process, ways to control the expression of Rubisco transgenes, the folding and assembly of Rubisco subunits in the chloroplast, and the regulation of the introduced enzyme. This research is a prelude to the ultimate goal of deciphering ways to effectively transplant more efficient Rubiscos into plants and how to get the enzyme to assemble and function appropriately. An overview of newly developed strategies for engineering foreign Rubiscos into plastids using a single transformation step will be presented.

10.13.5. Faster rubisco is the key to superior nitrogen use efficiency in NADP-ME relative to NAD-ME C₄ grasses

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In 27 C₄ grasses, photosynthetic and whole plant N use efficiency was greater in NADP-ME than NAD-ME species. This was due to lower N content in NADP-ME than NAD-ME leaves because neither assimilation rates nor plant dry mass differed significantly between the two C₄ subtypes. Relative to NAD-ME, NADP-ME leaves had greater *in vivo* (assimilation rate per ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalytic sites) and *in vitro* Rubisco turnover rate (*k_{cat}*) (3.8 versus 5.7 s⁻¹ at 25 °C). In two NAD-ME and two NADP-ME grasses, 30% of leaf N was allocated to thylakoids. Soluble protein represented a smaller fraction of leaf N in NADP-ME (41%) than NAD-ME (53%) leaves, of which Rubisco accounted for one seventh. The majority (65%) of leaf N and chlorophyll was found in the mesophyll of NADP-ME and bundle sheath of NAD-ME leaves. The mesophyll-bundle sheath distribution of functional thylakoid complexes (photosystem I and II, and cytochrome *f*) varied among species, with a tendency to be mostly located in the mesophyll. In conclusion, superior N use efficiency of NADP-ME relative to NAD-ME grasses was achieved with less leaf N, and Rubisco having a faster *k_{cat}*.

10.13.6. Environmental and ecological aspects of CO₂ assimilation

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Rubisco is an inefficient enzyme with a low turnover number and a competing oxygenase activity that causes C₃ plants to lose up to 50% of carbon fixed. Inefficiency is greatest at elevated temperature and when CO₂ is low (e.g. in drought when stomatal conductivity limits CO₂ availability at the site of Rubisco). We hypothesised that the selection pressure for high specificity factor () will be greatest under hot and arid conditions. Values of for purified Rubisco from 24 species of C₃ species found in diverse habitats with a wide range of environmental growth limitations were determined. Consistent with our hypothesis, Rubisco specificity factors tended to be higher in species from hotter and drier environments and in hemi-deciduous or evergreen species (Galmés et al. 2004). Molecular structures of Rubisco from such species may be exploited to improve crop performance. If introduced into tobacco, *Limonium gibbertii* Rubisco would increase net photosynthesis by 29 %.

10.13.7. Underwater photosynthesis studies on a terrestrial species identify novel responses of gas exchange capacity and photosynthesis biochemistry to submergence

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Gas exchange is severely hampered when terrestrial plants are flooded. A straightforward way to reduce the shortages of oxygen and carbohydrates might be photosynthesis under water. Several terrestrial plants develop 'aquatic' leaves which show pronounced anatomical differences compared to the 'aerial' leaves. We investigated if this remarkable plasticity facilitates gas exchange capacity under water. The cuticle thickness of the flooding tolerant species *Rumex palustris* was reduced upon submergence, resulting in decreased diffusion resistance which greatly facilitated underwater photosynthesis. Furthermore, the photosynthetic machinery itself was affected by submergence. Rubisco contents were reduced upon acclimation to submergence, indicating a lower carboxylation capacity. Electron transport capacity was also reduced in these leaves, but not as strong as the change in Rubisco, indicating a substantial increase of the ratio between electron transport and carboxylation capacity upon submergence. This novel finding is striking since this ratio is believed to be very conservative.

10.14.1. Evolution in a community context: Multi-species selection on plant floral and defense traits

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As members of multi-species communities, plants simultaneously integrate direct and indirect interactions with herbivores, pollinators and other species. Thus, traits expressed by plants may be influenced by a variety of selective agents. This perspective is in stark contrast to studies that focus on pairwise interactions, i.e., how floral traits are acted on solely by pollinators and how defensive traits reflect selection from herbivores. Here, I describe experimental studies in which the community context of selection on plant floral and defense traits is explored. For example, costs of plant defenses against herbivores may affect the size and/or numbers of flowers produced, the amount of floral rewards, or the palatability of these rewards. In such cases, a defended plant may become more pollinator limited, and pollinator preferences may constrain the response to selection from herbivores for increased defenses. Similar indirect interactions with non-pollinator species may constrain the evolution of floral traits

10.14.2. Linking above and belowground multitrophic interactions: root herbivory affects aboveground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality

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Plants and insects are part of a complex multitrophic environment, in which they closely interact. However, most of the studies have been focused mainly on bi-trophic aboveground subsystems. We studied whether root herbivory by *Delia radicum* can influence the development of the leaf feeder *Pieris brassicae*, its parasitoid *Cotesia glomerata* and its hyperparasitoid *Lysibia nana*, through changes in secondary plant compounds. In presence of root herbivory, the development time of the herbivore and the parasitoid significantly increased, and the adult size of the parasitoid and the hyperparasitoid were significantly reduced. Interestingly the effects were stronger at low root fly densities than at high levels of root herbivory. Higher glucosinolate (sinigrin) levels were recorded in plants exposed to belowground herbivory, suggesting that the reduced performance of the aboveground insects was via reduced plant quality. Our results show for the first time that root herbivory, via changes in plant quality, can reduce the performance of an aboveground three trophic level food chain.

10.14.3. Arabidopsis-a model for molecular approaches in plant volatile biology

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Volatile compounds are important mediators in plant environment interactions. We employ the genetic and genomic resources of *Arabidopsis thaliana* to study the molecular basis of biochemical and physiological processes as well as ecological functions of constitutive and inducible plant volatile emissions with particular focus on terpene volatiles. Arabidopsis flowers emit a complex mixture of monoterpene and sesquiterpene volatiles. In a reverse genetics approach, we have characterized all genes out of a large gene family of 32 terpene synthases (TPSs) that are responsible for the floral volatile emission. Analyses of gene expression and promoter activities revealed a highly restricted TPS activity in particular floral organs, the stigma and the intrafloral nectaries. Given the high vulnerability of these tissues to microbial infections, the active biosynthesis of terpenes at these sites suggests a primarily antimicrobial defense function for protection of reproductive organs. Studies with TPS knock out lines and different ecotypes under laboratory and field conditions will shed further light on the role of terpene volatiles in Arabidopsis flowers.

10.14.4. Phytochemical early warning systems: induced resistance to herbivores in ramet populations of clonal plants

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Plants are constantly exposed to the threat of herbivore damage. Many species have developed inducible defence mechanisms to deter feeding animals after an initial attack has happened. Such inducible resistance is considered beneficial in environments with spatio-temporally variable herbivore pressures as the production of costly defence chemicals is avoided whenever they are not needed. In many plants the defence induction signal (or the defensive phytochemical) can be transported throughout the plant, thereby conferring resistance to damaged and undamaged plant parts. This phenomenon, termed induced systemic resistance may be of particular importance to physically connected ramets of clonal plants because it allows for the internal transmission of phytochemical warning signals over considerable distances, thereby giving ramets of clonal plant species potential advantages over non-clonal neighbours in the case of imminent herbivore attacks. Our experiments provide clear evidence for ISR in ramet populations of the clonal herb *Trifolium repens*, and they show that the systemic transmission of early warning signals is constrained by phloem flows.

10.14.5. Signals from the deep. Root induction alters shoot-induced direct and indirect defences in wild *Brassica oleracea*

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Induced responses in plants are known to occur in both roots and shoots. In their natural environment, most plants will be attacked at both organs simultaneously. Because signaling hormones as well as induced defence products are transported systemically through the plant, root and shoot-induced responses may interact. Eventually, these interactions may constrain optimal defence induction. In wild *B. oleracea* we found that application of jasmonic acid (JA) to the roots yielded a significantly different glucosinolate profile in the shoots compared to shoot-application. Larvae of the specialist herbivore *Pieris rapae* performed significantly worse on root-induced plants. Similarly, plants pre-treated with JA at their roots before they were damaged and treated with *P. rapae* saliva, produced significantly different volatile profiles than plants pre-treated with JA to their shoots. Root induction thus affects both direct and indirect shoots defence levels. As a consequence, the presence of root feeders can have a significant effect on the aboveground multitrophic interactions associated with plants, and, eventually, plant fitness.

10.14.6. Variation in olfactory cues used by bees in flower selection

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While it is known that flowers attract pollinators through the display of visual and olfactory stimuli, our findings suggest that the relative importance of these cues to bees selecting flowers varies with the

particular bee-flower association. Using multiple-choice behavioral experiments (in cages and field) conducted on both solitary and bumble bees, and GC-MS analyses of floral scents, we investigated which floral stimuli are most decisive in a bee's discrimination among flowers. In different species of pollen-specialist bees, females locate host flowers using a specific interplay of cues, which include odors that originate primarily from the androecium, nectaries, gynoecium, and/or petals, depending on the flower fragrance chemistry. In bumble bees visiting *Rosa* species, petal cues are essential in attracting bees, whereas androecial stimuli, particularly pollen odor, determine whether a bee lands. Integrating behavioral and chemical studies is crucial to uncovering the dynamic sensory interactions between bees and floral traits.

10.14.7. Induction patterns in a Brassicaceae evoked by herbivory of a sequestering insect: correlating glucosinolate concentration and myrosinase activity

N. Martin, C. Müller;

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The binary defence system of Brassicaceae consists of preformed substrates and hydrolytic enzymes, namely glucosinolates and myrosinases. Upon tissue disruption these separately stored compounds interact to form various mostly toxic products.

We tested induction reactions of *Sinapis alba* following herbivory of larvae of the turnip sawfly *Athalia rosae*. Different developmental stages of larvae fed on young leaves of mature plants for 24 hours. Changes in primary and secondary metabolites were recorded in the damaged leaf as well as in the adjacent leaves and stems for the subsequent four days.

As these larvae sequester intact glucosinolates in their haemolymph, we determined plant glucosinolate concentrations as well as myrosinase activity.

Organ and time specific induction patterns upon herbivory were visible. These patterns differed according to the age of the feeding larvae and compound viewed. We assess whether leaves show stronger induction effects than stems and whether stems compared to leaves are defended via higher constitutive levels.

10.15.1. Biodiversity and ecological processes in tropical rain forests in the light of global land use change

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Habitat destruction and fragmentation constitute another major global environmental change and its current (and future) impact is likely to be of greatest significance in tropical ecosystems, where a large proportion of the Earth's biodiversity is concentrated. Most of the available information regarding habitat deterioration is based on rates. These, although inconsistent among researchers, suggest that annual habitat loss is in the order of 10-20 million ha. However impressive these estimates do not consider that the remaining habitat consists of forest fragments, isolated and affected by edges, to varying degrees. In addition, deforestation and fragmentation may synergistically interact with other anthropogenic impacts (e.g. hunting or climatic change). In this symposium we review current information regarding the impacts of deforestation/fragmentation and their synergisms with other disruptions in terms of: I) first-order effects, as determined by changes in species composition and abundance; II) functional aspects, concerning alterations of the performance of individual species, and III) higher-order effects, in terms of disruptions of the ecological processes in which biodiversity participates, including cascading extinctions at local levels. Speakers in this symposium will illustrate examples that make it evident that, even though local and global extinctions are a matter of concern from an evolutionary point of view, from a more functional perspective, we need to concern ourselves with the disruption of the functioning of individual components of biodiversity and with the extinction of ecological processes in tropical ecosystems.

10.15.2. Phenological study of the tree fern *Alsophila firma* (Baker) D.S.Conant in a Mexican montane forest

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Fifteen of 19 Mexican tree fern species are protected by Mexican laws (NOM-059-ECOL-2001), but there does not exist sufficient ecological information to elaborate efficient conservation strategies. We present the first phenological data set of *Alsophila*

firma (Baker) D.S.Conant. At the private montane forest reserve "Las Cañadas" (Huatusco, Veracruz, 19°10'35" N, 96°58'19" E, 1300 m a.s.l.), we observed a population of 31 individuals with a trunk of up to 2 m height. During one year, we measured monthly the length of the trunk and each leaf, and counted the number of living, fertile, new and dead leaves. From these data we calculated growth rates and leaf life span. *Alsophila firma* resulted to be a deciduous tree fern, shedding its pinnules from April to May at the beginning of the rainy season. Four to eight weeks later new leaves were produced synchronically. Leaves have a mean leaf life span of 10.0 ± 0.4 months. Individuals must be larger than 2 m to reproduce by spores, because only one individual became fertile. We estimate that at this site the oldest individuals of 9 m height will be at least 90 years old, because the mean annual trunk growth was 10.4 ± 1.4 cm.

10.15.3. Patterns of change in plant guilds during secondary succession in a tropical montane cloud forest area in Oaxaca, Mexico.

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Very little is known about the regeneration process of tropical montane cloud forest (TMCF) despite their ecological importance and the rapid disappearing of primary TMCFs on earth. We analysed the changes in composition and structure in five terrestrial plant guilds in three chronosequences each with 15, 45, 75, and >100 years after abandonment in Oaxaca, southern Mexico: We identified 209 species of plants distributed in 128 genera and 69 families. Each guild followed different patterns of change in density, species density (species / area), and richness across succession. Tree density was negatively correlated with shrubs, geophytes and low plants. As the age of the stand increased, the species richness, using rarefaction methods, and the species density of trees and lianas increased, but that of shrubs and low plants decreased. Except for trees and geophytes, the composition of abundant species in each guild was about the same. We conclude that the dynamics of each plant guild across succession should be considered in studies of vegetation dynamics and in programmes of forest management and conservation.

10.15.4. Cloud forest diversity of India

R. Srivastava, S. Biswas, R. P. S. Katwal;

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Forest biodiversity and genetic resources largely depend upon the water regime. Bioclimatic regions differ from one another on the basis of precipitation, evapotranspiration and other inter-dependent aspects of biological diversity. Cloud forest ecosystem of Indian sub-continent have been least explored. Two main features of such cloud forest ecosystem strikingly appear as year round flow of fresh water through the streams and endemic tree species with unique architecture of crown. In India such ecosystems are distributed in Shillong plateau in north eastern part, Little Andaman and Nicobar Islands in southern most part, Wenlock downs forests in Nilgiri Hills in western Ghats. Research need and priorities emphasize on (i) inventorization and characterization of the biodiversity using recent trends (ii) development of database and networking (iii) support and assistance for conservation planning and participatory approach (iv) capacity building and imparting education, and (v) integration of traditional knowledge into scientific and appropriate technologies.

10.15.5. Method to create an index to analyze the structure of vegetation

M. Diazgranados;

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A common problem in studies of structure is that there are many variables to analyze, and some times they suggest different interpretations. An index that represents the whole structure could be very helpful. I designed a method to create an Index of Structural Development (ISD), integrating horizontal and vertical structural aspects. Based in data of 34 variables sampled in 1084 trees of 14 diverse communities in an Tropical Humid Forest, after several filters and procedures (1st step: Barlett's and Scheffe's Tests, minimum squares; 2nd step: normality tests (K-S) and transformations (Box-Cox); 3rd step: Principal component analysis

(PCA) and Kaisers MSA) I chose five variables: total height (), leaf mass height (), first branch height (), dbh () and crown size (), that were the most representative. I run another PCA to estimate the eigenvectors of each variable. These values were standardized to obtain final balanced values (FV) for each variable. Finally, I constructed the index with this structure: $ISD = FV_1 + FV_2 + FV_3 + FV_4 + FV_5$. The ISD showed a normal distribution and it was very useful in comparisons between trees or between communities.

10.15.6. Biomonitoring of disturbance in neotropical rainforests using bryophytes as indicators

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BRYOMONITOR is a computer program for monitoring disturbance and regeneration of neotropical rainforests using bryophytes as indicators. The program has been developed using field data from different areas in Ecuador, Peru, Bolivia and Argentina. It can be applied in wide parts of the Neotropics between sea level and 2000 m altitude. The program is based on the examination of changes in species composition of woody plants and bryophytes in rainforests having various degrees of disturbance. The program uses 34 indicator species, of which 17 are characteristic for primary forests and 17 species are typical for secondary forests. Ten tree stems must be examined from the ground to 2m in height for the application of the biomonitoring system. The computer program for Windows-PC calculates a naturalness index for the forest based on the presence of the indicator species. An index of 10-9 characterises primary forests. Typical logged forests show naturalness indices between 7 and 5 and secondary forests have indices between 2 and 4. An index of 1 is characteristic for isolated trees.

BRYOMONITOR can be used free of charge. It is available at <http://www.drehwald.info>.

10.16.1. Medicinal plants research and development for sustainable health

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Health is considered as prerequisite for achieving the supreme ends of life and preventive and curative aspects of diseases are considered as important components of the concept of positive health. The traditional Indian system of medicine predominantly use plant based raw materials in most of the preparations and formulations. Modern pharmacopoeia also contain at least ¼ drugs derived from plants and many other which are synthetic analogues built on prototype compounds isolated from plants. Medicinal plants being natural non-narcotic, having no side effect offer a range of safe, preventive and curative therapies which could be useful in achieving the goal of "Health for all" in a cost effective manner. China and India are two great producers of medicinal plants having more than 40% of global biodiversity. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. in the national and international markets. Several constraints exist due to inadequate awareness, inadequate investments in research and development, lack of quality and standardization norms etc. Recent developments in propagation and bioengineering of medicinal plants leading to value addition, conservation and genetic diversity, quality standardization, focused research and development on products lines, industry linkage etc. will be presented.

10.16.2. The usage of wild medicinal plants, berries, fruits and mushrooms in taiga area of Russia

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The list of wild medicinal plants species, the average crop and the peculiarities of usage of the main species of wild berries, fruit plants and edible mushrooms in 2000-2003 are defined using the methods of questionnaire and natural investigations. It is determined that there are 227 species of medicinal plants, 27 species of fruits and berries, 250 species of edible mushrooms. In general, the stocks of medicinal plants in Kirov region have been being used for not more than 3,2% from possible stocking up volumes. Protected species: *Centarea sumensis* Kalen., *Calypso*

bulbosa (L.) Oakes, *Paeonia anomalia* L., *Pulsatilla patens* (L.) Mill are often used as medicinal plants, especially in public medicine. The populations of this species are decreasing because of such influence. The increased usage of the resources of all studied species is noticed: berries and fruits - 5 times, edible mushrooms - 2 times more intensive compared to the level of 1980's.

10.16.3. Ascertaining the genetic variability of *Andrographis paniculata* Nees in Tirunelveli hills in Tamilnadu in India

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Kalmegh (*Andrographis paniculata* Nees) belonging to the family Acanthaceae, is one of the most important herbs used in Indian traditional Ayurvedic and homeopathic system of medicine for treatment of liver disorders as a febrifuge, alternative, jaundice, worms, diarrhoea, dysentery and digestive complaints. It is also a blood purifier. The drug is mainly recognized for its diterpenoids - andrographolide and related compounds. Genetic improvement for quantitative traits in Kalmegh can be achieved through a clear understanding of the nature and amount of variability present in genotypes and the extent to which the desirable traits are heritable. In order to ascertain the genetic variability, plants were collected from different populations at various localities and Random Amplified Polymorphic DNA (RAPD) fingerprints were analyzed by Polymerase Chain Reaction (PCR) of genomic DNA using random primers. The RAPD fragments were scored for presence/absence to calculate Jaccard's similarity index. Clustering based on similarity index was done following unweighted pair group with arithmetic mean method and a dendrogram was constructed and analyzed.

10.16.4. Ethnomedicinal plants and their indigenous uses to cure arthritis at Khunti, Ranchi district (Jharkhand), India.

M. Mahato, R. Chandra, J. Kumar;

Ethnobotanical and Medicinal Plants Laboratory, Ranchi University, Ranchi, Jharkhand, India.

The term arthritis describes a range of readily diagnosable inflammatory conditions of the joints. The classification, diagnostic criteria and treatment approaches to these conditions have all been established to a greater or lesser extent in the international literature. However it is a common disease found frequently in all part of the world. The traditional knowledge of plant based remedies are said to be very useful to cure this disease. Authors carried out an ethno-botanical survey in the above-mentioned area for herbal medicine used in arthritis. The information is based on the exhaustive interview with local herbal practitioner and others who prescribe their own herbal preparation. During ethno-botanical survey, 70 plants belonging to 42 families have been reported from the study area used by the herbal practitioner of that area. Importance was given on their herbal formulation and mode of administration so that in future it's scientific evaluation and pharmacological validation can be done.

10.16.5. A comparative study of the ethnomedicinal plants of Maharashtra in India and the Northern Provinces of Thailand

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The presenter of this paper is the author of three books on medicinal plants and a Professor of Botany and Anthropology. He has carried out over 25 botanical and anthropological field expeditions among the tribal communities of Maharashtra in India and in the northern provinces of Chiangmai and Chiangrai in Thailand. In a powerpoint presentation this extensive work on the ethnomedicinal plants that are often administered by recognized magico-religious leaders or shamans brings to light interesting similarities and differences in the way medicinal plants are used by people in the two countries. The presentation covers the description and classification of the local diseases, the plants and the parts used, the medicinal use, the dosage and caution, if any. The Scope in the field of ethnomedicine in both countries is really great, especially for ethnobotanists, pharmacists and pharmaceutical companies.

10.16.6. Evaluation of antiperoxidative and anti-inflammatory property of a herbal formulation "Arthosafe" in adjuvant induced arthritic rats

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¹Dr.R.Baskar, Coimbatore, India, ²Dr.G.R.Damodaran College of Science, Coimbatore, India.

Rheumatoid arthritis is an autosomal disorder characterised by non-specific chronic inflammatory disease of the joints. The tissue damage might be due to the liberation of reactive oxygen species (ROS) from phagocytes invading the inflammatory tissues, which reacts with membrane lipids resulting in highly damaging reaction. The present study focus on the combination of four medicinal plants - *Acalypha indica*, *Coriander sativum*, *Ricinus communis* and *Zingiber officinalis* as an anti-inflammatory agent. 4 groups of 6 male rats were divided as Group I-normal control, Group II- injected 0.1 ml of Freund Complete Adjuvant, Group III- administered herbal extract for 30 days, Group IV- adjuvant induced rats treated with extract for 30 days from day 21. As per observations, a significant increase in lipid peroxide with a simultaneous decrease in antioxidant levels were noticed in arthritic induced rats compared to controls. Supplementation with the herbal drug formulation restored the levels of lipid peroxides and antioxidants in treated rats. These observations suggests the efficacy of the herbal formulation as an antiperoxidative and anti-inflammatory agent.

10.16.7. Endemic and endangered medicinal plants of eastern ghats of India**N. Savithramma;**

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Eastern Ghats (EG) are one of the megacentres of endemism in India. The EG are the source of endemic plant gene pool. Tirupati-Cudapah-Nallamalai hills are the 11 th Hot spots of endemism of EG. The geological factors of this forest area is cudapah formation and soil types with red and calcareous shales. The vegetation supports edaphic endemics like valuable *Pterocarpus santalinus* and *Santalum album* and narrow endemics like *Boswellia* type and *Calamul* type. Endemic Flagship species like *Actinodaphne madraspatana*, *Shorea tumbuggaia* Keystone endemic species of *Memecylon* and other highly important medicinal endemic species like *Pimpinella tirupatiensis*, *Lucas lavandulaefolia*, *Boswellia ovalifoliolata*, *Decaschistia cudapahensis*, *D. crotonifolia*, *D. rufa*, *Cassia montana*, *Aglaiia elaeagnoides*, *Indigofera barberi*, *Rhynchosia beddomei*, *Cycas beddomei*. Southern parts of the hills are harbouring local endemics like *Andrographis nallamalayana*, *Eriolaena lushingtonii*, *E. hookeriana*, *Crotalaria madhurensis*, *Nilagirianthus circarensis*. The gene pool niches will be discussed.

11.1.1. Chimeric studies on the organization of the shoot apical meristem**R. W. Korn;**

Bellarmine University, Louisville, KY, United States.

Eversporting chimeras provide an excellent opportunity for the analysis of the shoot apical meristem (SAM). Data from seven species of Cupressaceae indicate green-yellow chimeras on wild type green shoots produce stable yellow shoots 82-92% of the time. This information best supports the idea that a single apical cell in a yellow tunica undergoes a replacement periclinal division (Dermer's concept) to give one daughter cell in the corpus. The chimeric result of this division is then explained as an asymmetric deployment of the yellow clone produced from the original yellow sister corpus cell. Similar results were found for three spiraea varieties, indicating that some, if not all, dicots also have an apical cell in SAM. Computer model studies on growth rates in various regions of SAM give an apical and stable dome only when the growth rate is highest at the apex and decreases with distance from this site. Also, the green-yellow chimeric shoot regions range from two to 15 nodes in length and the data follows a gamma distribution, suggesting the growth just below SAM where leaves form is far more active than above at the stem apex.

11.1.2. Stem cell niches in plants

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The stem cell populations of shoot and root meristems are reliably maintained although cells continuously leave the meristem and are replaced by new ones. We previously showed that the stem cells in shoot meristem are controlled by signals from an underlying organizing center, expressing the WUSCHEL gene (Cell 95, 805-815), and that the size of the stem cell population is dynamically

regulated by a feedback loop between stem cells and organizing center (Cell 100, 635-644). This signaling circuitry appears to have the potential to act as a self-regulatory system that is integrated into a larger regulatory network to control organ formation from the shoot apex (Development 129, 3195-206; *ibid.* 130, 3163-3173). In the root meristem, stem cells maintenance requires short range signaling from the quiescent center (Scheres group, Nature 390, 287-289). Here we discuss commonalities and differences between the stem cell niches in both apical meristems.

11.1.3. Involvement of the Arabidopsis ET family in cell differentiation

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EFFECTORS OF TRANSCRIPTION (ET) are plant specific proteins characterized by 2-4 C-terminal DNA and zinc binding repeats with a highly conserved cysteine pattern. Over-expression in tobacco results in phenotypic changes including dwarfism, reduced xylem lignification, ectopic anthocyanin synthesis and poor germination rate. Transient expression studies on gibberellin (GA)-regulated gene promoters and feedback induction of GA synthesis genes in ectopic over-expression lines suggest that ET factors function as modulators of GA-responses.

Here we report an initial characterisation of the three-member gene family found in the *Arabidopsis* genome. Mutant analysis indicates that ET factors are involved in the control of cell differentiation interacting with KNOX-like (KNAT) genes as well as controlling the expression of the GA-induced cell cycle regulator GASA4. The action of ET is topologically restricted by a mechanism preventing them from entering the nucleus in non-differentiating cells.

11.1.4. Towards a model for organ initiation patterns at the shoot apical meristem

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During the last decade an impressive body of knowledge concerning shoot meristem function has been generated. The complexity of these data is such, that an integrated view of meristem function is not possible. Therefore, adapted mathematical and informatics approaches are now required to integrate the knowledge. Here we will present the first results obtained on the mathematical modelling of auxin fluxes in the meristem. Auxin is actively transported from cell to cell. Polar localised membrane proteins of the PIN1 family facilitate this transport and recent hypotheses suggest that auxin maxima created by these proteins are at the basis of organ initiation. To further test this concept, we have analysed the patterns of PIN1 distribution at the shoot apex using specially designed modelling tools. The results not only confirm that this distribution is compatible with existing hypotheses, but, in addition, they reveal novel properties of the PIN1 network. In particular they suggest an important role for the central zone at the meristem summit in the re-distribution of auxin. Experimental data to test the implications of the computer simulations will be presented.

11.1.5. Leaf dissection: Growth analysis and genetics

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Leaves can be simple and entire, or they can be dissected. Leaf dissection is the process of elaborating the margin of leaves into segments such as teeth, lobes and leaflets. The portion of the leaf margin competent for fractionation into segments is the marginal blastozone. One approach to understanding the interplay of gene networks and leaf dissection is to analyze mutants for differences in growth, development and gene expression patterns. Growth analysis of mutants in dissection depth reveal alterations in: timing of initiation, initial size, and relative growth rates. Mutants altering complexity of dissection appear to act through controlling the duration of competence of the marginal blastozone. There is neither a universal developmental mechanism or gene for altering dissection depth, nor a common gene network for the complexity of dissection. This indicates multiple and possibly redundant genetic pathways that specify dissection in angiosperm leaves.

11.2.1. genetic and proteomic analysis of gravity signal transduction in *Arabidopsis* roots

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Arabidopsis roots respond to gravistimulation by developing a curvature that is modulated by a lateral gradient of auxin. This gradient originates in the columella statocytes through lateral repositioning of the PIN3 auxin efflux facilitator. We used genetics to identify proteins that contribute to gravity signal transduction. ARG1 and ARL2 are needed for lateral auxin transport across the cap. ARG1 is associated with the vesicular trafficking pathway, suggesting it regulates PIN3 function or trafficking. Genetic modifiers of *arg1-2* were obtained and shown to enhance the gravitropic defect of *arg1-2*. The corresponding proteins function in interpretation of the gravity-vector signal. Furthermore, a proteomic approach allowed identification of root-tip proteins that are differentially represented in response to gravistimulation. Subsequent reverse genetic studies demonstrate a role for adenosine kinase and the AdoMet pathway in gravity signal transduction (This work was supported by grants from NASA and NSF).

11.2.2. Signal transduction pathway for gravitaxis in the flagellate *Euglena*

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A recent experiment on a sounding rocket comparing immobilized *Euglena gracilis* cells with mobile cells demonstrated that a passive buoy effect can account for no more than 20 % of the orientation of the cells in a gravity field. The cells show either positive or negative gravitaxis depending on other external or internal factors. The negative gravitaxis of older cells is inverted into a positive one by stress factors such as increasing salinity or exposing the cells to excessive visible or UV radiation mediated by reactive oxygen species (ROS). The primary reception for the gravity vector seems to involve mechanosensitive ion channels which specifically gate calcium ions inwardly. The influx of Ca²⁺ activates calmodulin which has been shown to be involved in the sensory transduction chain of graviorientation. In addition, adenylyl cyclase is involved; it produces cAMP which has also been shown to be the key messenger in phototaxis in the same organism mediating flagellar reorientation and thus course correction of the swimming path.

11.2.3. How to activate a plant gravireceptor

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Plant gravity sensing involves controversially discussed mechanisms by which higher plants translate the physical signal from amyloplast (statolith) sedimentation into a physiological signal. Gravisensing cells of characean green algae, rhizoids and protonemata, were established as model systems for studying specific aspects of gravitropism including the early gravisensing mechanisms. Experiments performed on ground and in microgravity have demonstrated that actin and myosin are key players in the process of gravity sensing. Actomyosin forces keep statoliths in a position of balance, in which they compensate the effect of gravity and they provide a guiding system that directs sedimenting statoliths to specific graviperception sites on the plasma membrane, where gravireceptors are activated that initiate the graviresponse. Gravisensitivity, therefore, is basically determined by molecular interactions between statoliths and actomyosin. Parabolic flight experiments promise to disclose the mechanisms of gravireceptor activation in higher and lower plants.

11.2.4. Gravity-regulated oxygen uptake in root apices

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Using two O₂-selective microelectrodes, operated non-invasively in self-referencing mode to simultaneously measure oxygen fluxes in different regions of the root apex, we observed gravity-regulated asymmetric oxygen influxes changes in the transition zone. The kinetics of these changes have been studied on ground and under

changing gravitational conditions during parabolic flights. On ground, approximately 20 s after changing the position of the roots from the vertical to the horizontal, oxygen influx increase selectively on the physically upper root flank of the transition zone and remained stable on the lower flank. The oxygen asymmetry appeared 18 ± 2 s (mean ± SD) after repositioning. Taking into account the tilting procedure (around 15 s), the first signal can be hypothesized to appear few seconds after stimulation. During parabolic flights we detected an enhancement of the oxygen influx already after 3.5 ± 0.3 s of attaining the microgravity conditions. This rapid change in the oxygen flux into root apices is by far the fastest ever reported plant response to gravity. The significance of these results on the nature of the graviperception will be discussed

11.2.5. The PIN auxin efflux facilitator network controls growth and patterning in roots and shoots of *Arabidopsis*

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The plant hormone auxin has long been known to play a crucial role in plant growth and development, but how it affects so many different processes has been a mystery. Recent evidence from genetic and molecular studies has begun to reveal a possible mechanism for auxin action. We will present an overview on auxin's role in roots and shoots. Local accumulation of the plant growth regulator auxin mediates pattern formation in *Arabidopsis* roots and influences outgrowth and development of lateral root- and shoot-derived primordia. It was unclear however how auxin can simultaneously regulate patterning and organ outgrowth and how its distribution is stabilized in a primordium-specific manner. We show that the PIN auxin efflux facilitator genes collectively control auxin distribution to regulate cell division and cell expansion in the primary root and shoot. It is widely believed that the PIN proteins are crucial for proper cellular co-ordination. This family of auxin efflux facilitators occurs in monocotyledonous and dicotyledonous plants. We will summarize their evolutionary history, expression profiles, and, where appropriate, relating them to protein function. The joint action of these genes has an important role in pattern formation by focusing the auxin maximum and restricting the expression domain of other genes.

11.2.6. Gravity stimulation induces fast and transient transcriptional regulation in roots.

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Plant roots sense changes in the vector of gravity which ultimately leads to a differential growth response. To understand the regulation of gene expression involved in gravitropism, we carried out a time-course analysis of whole genome transcript changes throughout the first hour of gravitropic and mechanical stimulation in the *Arabidopsis* root apex using microarray technology. Our results indicate both the relative abundance and timing of events are important for establishment of gene expression cascades for either stimulus. Significant changes in transcriptional regulation occurred throughout the first hour of gravity and mechanical stimulation in diverse gene families coding for transcription factors, cell wall modifying enzymes, transporters, protein kinases and phosphatases, hormone metabolism and cell cycle proteins. Functional characterization of a cluster of fast and transiently regulated gravity specific genes will give new insights into signal transduction elements and pathways involved in root gravitropism and their integration with other stress responses.

11.2.7. SGR5 encoding a zinc finger protein involved in gravitropism of *Arabidopsis* stem

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Plant can change the growth orientation after sensing the gravity orientation. To elucidate molecular mechanisms of the gravitropism, we have characterized abnormal shoot gravitropic mutants (*sgr*) of *Arabidopsis*. We have shown that 1) endoderm cells in the inflorescence stems are graviperception sites, 2) amyloplasts sedimentation in these cells is important for gravity perception and 3) amyloplasts movements are controlled by the

vacuolar membrane dynamics. The *sgr5-1* showed reduced gravitropism only in inflorescence stems, in which amyloplasts sedimented normally. The *SGR5* encodes a novel zinc finger protein with coiled-coil motif. The functional *SGR5-GFP* protein located in the nucleus, suggesting *SGR5* may function as a transcription factor. The *GUS* driven by *SGR5* promoter is expressed in several tissue layers including endoderm cells. When *SGR5* was specifically expressed in the endoderm of *sgr5-1*, transgenic plant showed normal gravitropism. These data indicated that *SGR5* might be involved in the gravity perception, signal formation and/or signal transduction process in the endoderm cells after amyloplasts sedimentation.

11.3.1. Bilabiate flowers: the ultimate response to bees?

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Bees are by far the most important pollinators as they have to nourish offspring, especially with pollen. Therefore, they must visit many more flowers than do all other pollinators. As bees try to maximize pollen harvest, however, they often leave nothing for pollination. To reserve pollen for its original purpose, flowers should offer nectar or other non-pollen attractants, hide pollen from early perception and place it out of immediate reach of the (fore)legs generally used in pollen uptake. Hiding pollen below bees as in keel blossoms requires mechanically complex flowers, as all legs have to be kept busy. Locating pollen on top of the visitor is much more advantageous: Inside a dorsiventral flower, a closely fitting upper lip impedes access, and in flight, when grooming and reloading usually occur, the beating wings make pollen unavailable. Bilabiate flowers are obviously an optimal adaptation to (and against!) bees, they evolved in many angiosperm groups, especially the Lamiales. Originating from this obvious key innovation, alleys were opened up to more sophisticated flowers and new pollinators, as will be shown in the presentation.

11.3.2. Evolution in the genus *Salvia*; species radiations, floral innovations, and biogeography

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The genus *Salvia* has traditionally included any member of the tribe Mentheae (Lamiaceae) with only two stamens, and with each stamen expressing an elongate connective. Our data suggests an independent origin of the elongate connective on at least two different occasions: once in the New World, and at least once in the Old World. We investigate the elongation of the connective tissue in members of tribe Mentheae from a phylogenetic standpoint, characterize staminal morphology in each radiation, and address the implication of the non-monophyly of *Salvia* on the manner in which the lever-mechanism of pollination has originated in *Salvia*. Associated with each major radiation of *Salvia*, we can document the following progression: abortion of two of the four stamens, swelling of the connective tissue, slight elongation of the connective tissue separating the thecae, further elongation of the connective tissue resulting in two much separated thecae, but no lever mechanism, and finally, abortion of the posterior theca and employment of a lever mechanism. Significant species radiations have only occurred in those lineages employing a lever mechanism of pollination.

11.3.3. Structure of inflorescences in the genus *Salvia* L. (Lamiaceae)

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The structural analysis of flower arrangement in *Salvia* important for taxonomy, phylogenetics and floral biology has been performed. About 600 species were studied, 44 of them were cultivated in Central Siberian Botanical Garden in Novosibirsk, Russia. Two basic variants of floriferous axial systems which are synflorescence and intercalary florescence were established. Structure of synflorescences in various *Salvia* species was determined by the branching order of axes and by the number of floral units. The floral unit in studied species was always compound and indeterminate, it was thyrsus, sometimes reduced to botryoid one. Floral leaves were usually present but

occasionally were deciduous. Intercalary florescences of two types were revealed only in the subgenus *Calosphace*. The first one was floriferous zone homologous to thyrsus, with the reduced cymes as lateral subunits. The second type was intercalary double raceme. The main trends of evolutionary transformations of inflorescence are the following: reduction of metamers of axes, reduction of a number of elements, polymerization of subunits and formation of brightly coloured enlarged bracts.

11.3.4. Floral diversity and pollen transfer in bird pollinated *Salvia* species

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Among the more than 900 *Salvia* species about 250 are bird pollinated. With few exceptions, they occur in the New World. They belong to five different sections and show a high floral diversity. The staminal lever which ensures precise pollen transfer in most bee pollinated species by its easily going reversible movement is strongly influenced by the transition from bee to bird pollination. In many ornithophilous species the staminal lever is functional, but in some it is restricted or completely reduced. Diverse modifications of the lever and their consequences for the pollen transfer by birds are illustrated. They range from narrow corollas which leave no space for any lever movement to immobile levers due to a stiff or even lacking joint and completely reduced lower connective arms. It is assumed that the driving force behind the diverse modes of reduction is the necessity to enlarge the distance between nectar and pollen, thereby ensuring pollen deposition on the birds' feathered head. This is achieved by corolla elongation and/or exposition of the pollen-sacs. As soon as pollen is freely accessible the lever movement loses its significance for pollination.

11.3.5. Pollination in the Australian endemic genera *Hemigenia* R.Br. and *Microcorys* R.Br. (Lamiaceae): the role of staminodes and mobile anthers.

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The fertile stamens of *Hemigenia* R.Br. and *Microcorys* R.Br. have enlarged anther connective tissue. The upper end of the anther bears a fertile theca, and the lower end may be bearded or bear a reduced theca. The entire anther is mobile on the filament. Insects visiting the flowers push the lower end of the anther to access nectar and in doing so lever the upper end onto their head or back. The bearding on the lower ends of the anthers catches adjacent anthers so that they lever in unison towards the pollinator. The flowers are protandrous. The mature style touches the pollinator in the same place as the fertile thecae. The mobile anthers combined with corolla shape provide precise pollen deposition and therefore effective mechanical isolation. In some species, the abaxial stamens are sterile, and the anthers are reduced to club-like lobes. These staminodes protrude from the lower corolla lip and guide the insect into the throat. Observations of pollination in the wild have demonstrated isolation by exclusion of inappropriate size pollinators, and pollination by insect castes defined by size and feeding mode.

11.3.6. Floral design and pollination syndromes in southern African species of *Plectranthus* (Lamiaceae)

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Amongst the ca. fifty species of *Plectranthus* that occur in southern Africa, three main pollination syndromes have been described. A pollination syndrome involving the long-proboscid nemesinid fly, *Stenobasipteron wiedemanni* (with proboscides up to 30 mm long), operates in species with long corolla tubes that occur in forest habitat along the eastern seaboard of the region. Another group of species with sigmoid-shaped corolla tubes is pollinated by medium-proboscid nemesinid flies and long-proboscid bees (with proboscides up to 10 mm long) that are able to access nectar with flexible proboscides. The remaining shorter-tubed species are pollinated by a group of generalist bees and flies, amongst which acrocerid flies (*Psilodera* spp.) are of particular interest due to their intermediate position in relation to corolla tube length. Corolla shape and size, as well as habitat preference, largely determine which suite of pollinators are utilised by species of *Plectranthus*. This paper outlines adaptations in floral design in response to pollinator type.

11.3.7. Floral characteristics, pollen viability, and stigma receptivity in two gynodioecious protandrous species (*Teucrium capitatum* and *Origanum syriacum*), (Lamiaceae).

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Testing the relationship between stigma receptivity and pollen viability in *Teucrium capitatum* L. and *Origanum syriacum* L. we found three plant types in each species: plants bearing hermaphrodite flowers or male fertile flowers (MF), plants bearing female or male sterile flowers (MS), and intermediate plants bearing flowers with different degrees of pollen abortion (INT). Plant types differed in flower size, with the MS flowers being shorter than the other two types. There was no difference in style length between plants types in *T. capitatum* did not differ in style length. Stigma receptivity increase over the lifetime of the flower followed different models of curves (logarithmic in MS and INT flowers with a slight tendency to a linear model in INT, and exponential and cubic in MF flowers from *Teucrium* and *Origanum* respectively). Pollen viability steadily declined with flower age and their relationship clearly showed a female phase totally conditioned by a male phase. Each species followed different strategies to avoid pollen-stigma interference but followed the same strategies to avoid "pollen clogging".

11.4.1. Dating the origins of plants endemic to the Corso-Sardinian microplate: A window on the biogeography of the western Mediterranean basin

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Corsica and Sardinia, the largest islands of the W Mediterranean, have been identified as two of the areas with the highest species richness in the Mediterranean basin, hence they play a key role for understanding Mediterranean biodiversity. Furthermore, the well-known geologic history of the Corso-Sardinian microplate, marked by the Late Oligocene split from northeastern Spain and southern France, and proposed land bridges with Tuscany during the Miocene and northwestern Italy during the Pleistocene, provides the necessary framework to investigate the relative contribution of land connections and over-water dispersal to the assembly of the endemic flora. The origin of a selected group of Corso-Sardinian endemics is being investigated through a combination of phylogenetic analysis, molecular dating, and ancestral area reconstruction. After a review of the geologic history of the Corso-Sardinian microplate, preliminary results will be presented on the origin of *Ruta corsica* (Rutaceae), eight endemics in the Boraginaceae, and three endemics in the Araceae.

11.4.2. Plant hybridization in the western Mediterranean basin: disturbing forces for species cohesiveness or key inputs for plant speciation?

J. A. Rosselló;

Jardí Botànic, University of Valencia, Valencia, Spain.

The Western Mediterranean basin is one of the rich speciose sites within the whole Mediterranean hotspot and includes many endemic taxa showing a restricted distribution. For a long time it has been assumed that most of this plant diversity has been shaped by geographical and ecological patterns. Geographical variability in several Mediterranean species has been usually treated as noise when dealing with taxonomic treatments, since the peripheral populations of several allopatric species may sometimes be linked by a mosaic of morphologically intermediate, but geographically still discontinuous, non-interbreeding and long-isolated populations. However, increasing evidence shows that interspecific gene flow (without genome duplication through polyploidy) has been of great importance in driving cryptic speciation in several groups. In this talk, we will show that speciation by geographic isolation and a further superimposed pattern of complex gene flow among narrowly endemic taxa and widespread species has been involved in the diversification of this Mediterranean flora. This work has supported by funds of the project REN2001-3506-CO1-01.

11.4.3. Models for reticulate evolution in Mediterranean lineages: Does *Armeria* break molds?

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Reticulate evolution in *Armeria* has been documented using several sources of evidence including nuclear ribosomal DNA and non-coding chloroplast DNA sequence data, among others. Some patterns of molecular variation discovered by our team in this genus and the inferred responsible mechanisms were eventually considered extraordinary. These include a species-independent geographical structure for ITS variation, the mechanisms proposed to account for such structure (extensive hybridization and biased concerted evolution of these regions) and the phylogenetic signal found in the same ITS data set. Other features also detected in *Armeria* were considered unusual, such as the sharing of chloroplast haplotypes by different species in the same massifs, the species-independent geographical structure for chloroplast sequence data, the highly variable intraspecific RAPD profiles, or the inferred compilospecies model of evolution. Recently published and ongoing studies particularly in the Mediterranean on other plant groups have questioned the rarity of such patterns associated to reticulation.

11.4.4. Phylogenetic patterns and polyploid evolution within the Mediterranean genus *Centaurium* (Gentianaceae - Chironieae)

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The genus *Centaurium* (Gentianaceae) comprises ca. 27 annual species and subspecies primarily distributed in the Mediterranean basin. Phylogenetic relationships, inferred from sequences of both nrDNA (internal transcribed spacer, ITS) and cpDNA (*trnL* intron and *trnL-F* spacer) regions, confirm the monophyly of *Centaurium* and reveal the importance of polyploidy within the genus. In each ITS clade, diploid to hexaploid species of *Centaurium* occur. Furthermore, polyploidization is often associated with hybridization events, as suggested by the importance of additive polymorphic sites detected in the ITS region. Hence, reticulation appears to be the main cause of phylogenetic incongruence and resulting systematic controversies detected in the genus. Based on molecular and cytological evidences, the origin of some polyploid systems is discussed. Allopolyploidy associated or not with introgression, appears to be the main evolutionary trend in *Centaurium*, and only a few autopolyploid taxa have been detected so far.

11.4.5. Evolution of Mediterranean Malveae: molecular phylogenetics, ploidy level and genome size

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Mediterranean Malveae include species of *Malva*, *Lavatera*, *Malope*, and *Althaea*. All of them except one (*L. trimestris*) belong to a lineage of polyploids where *L. phoenicea* and the genus *Malope* are placed in basal positions. Within this natural group, molecular phylogenetic analyses based on nrDNA (ITS) and cpDNA sequences (*trnL-F* + *psbA-trnH*) reveal the presence of several natural lineages incongruent with classical taxonomy e.g. the Malvoid group, the Lavateroid group, the *M. moschata* group, and annual altheas. The Malvoid group, supported by cpDNA and nuclear evidence include two lineages: one Macaronesian-W Mediterranean and another mostly Temperate-Mediterranean which extends to *Lavatera* from E Africa, Australia and California. In this group ploidy level ranges from 6x-12x, but genome size only correlates with ploidy among annual herbs. The Lavateroid group has morphologically diversified in W Mediterranean preserving a low nucleotide divergence in cpDNA sequences, ploidy level (6x) and genome size. The uncertain placement of *L. trimestris* (2x), and *M. hispanica* (4x), incongruent between chloroplast and nuclear phylogenies, is discussed.

11.4.6. Evolutionary history of the Aegean *Nigella arvensis* alliance (Ranunculaceae)

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Phylogenetic, phylogeographic and population genetic analyses were used to elucidate the evolutionary history of the Aegean *Nigella arvensis* alliance based on ITS sequence and cpDNA haplotype variation. The study revealed three major cpDNA lineages in the Western, Central, and Eastern Aegean that likely evolved *in situ* from a pan-Aegean ancestral stock as a result of fragmentation events, triggered by post-Messinian sea flooding, Pleistocene eustatic changes and corresponding climate fluctuations. Founder events played an insignificant role in the group's history. Rather, the alliance is identified as a poor seed disperser prone to genetic drift. In particular, observed levels of cpDNA differentiation *between* Kikladian island populations (*N. degenii*) largely reflect Holocene island fragmentation and genetic drift in the near absence of seed flow since their time of common ancestry. The phylogeographic history inferred for the alliance conforms remarkably well to previous hypotheses about the dominant evolutionary processes driving plant evolution in the Aegean: past fragmentation and genetic drift coupled with restricted gene flow.

11.4.7. Phylogenetic biogeography of Crassulaceae: comparing patterns of lineages endemic to Macaronesia and southern Africa

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Crassulaceae are a medium-sized family of leaf-succulent herbs and shrubs. The family is geographically widespread, but discrete centers of diversity occur in southern Africa, Macaronesia, Mexico/South America, and Asia. Phylogenetic analyses of the family based on cpDNA RFLPs and *matK* sequences are congruent and recover six major clades. Lower-level phylogenetic studies employing broad taxonomic sampling and extensive molecular data have been conducted on two of these lineages: the Macaronesian clade and the southern African genus *Crassula*. It is possible to use these phylogenies to compare biogeographic patterns and dispersal events among the species comprising these insular and continental lineages. Among species in the Macaronesian clade dispersal between islands into similar ecological zones is common, with radiation on a single island occurring only rarely. Thus, many of the clades comprise species that are geographically widespread, yet morphologically similar. This same pattern occurs among species of *Crassula*; however, several clades within *Crassula* contain morphologically diverse species that are geographically cohesive.

11.5.1. Basidiomycetous phylogeny seen through the electron microscope

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Because of some inconsistencies in molecular phylogenetic hypotheses, ultrastructural characteristics continue to play an important role in interpreting the evolution of basidiomycetes. In this talk, significant ultrastructural characteristics of these fungi, such as septal pore apparatus, spindle pole bodies, symplectosomes, colacosomes as well as myco- and phytoparasitic cellular interactions etc. will be discussed with a focus on their role as phylogenetic markers. Interestingly, the basal basidiomycetous groups are especially rich in features at the ultrastructural level. Furthermore, septal architecture and mode of cellular host-parasite interactions varied in the ustilaginomycetous history, whereas septal pore caps developed in some variations in the hymenomycetous evolution. From the ultrastructural point of view the obvious questions to address are the following: are the Urediniomycetes and the Atractiellales monophyletic? Is *Cryptomycocolax abnorme* the most basal basidiomycete? Was mycoparasitism the fundamental motor in the early basidiomycete evolution?

11.5.2. Quantification and characterization of substrate mycelium by microscopy

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The biomass of fungal mycelium within the soil is of particular interest, when ectomycorrhizal fungi have to be considered as an important sink of tree carbohydrates. Between 15 and 30% of the

net photosynthesis products are estimated as the investment into the symbionts for adequate tree growth. Some biochemical markers have already been applied (ergosterol and phospholipid fatty acids) combined with a subtractive design to calculate the living mycelium of ectomycorrhizae. We try to estimate the ectomycorrhizal mycelium by microscopy, combining image analysis with a calibration with TaqMan Real Time PCR of the extramatrical mycelium of synthesized ectomycorrhizae. We show that ectomycorrhizae of different exploration types can differ greatly in their mycelial biomass, their organization, and their exploiting range. Given that the mycelium biomass of *Tylospora asterophora* is set to 100 %, *Piloderma croceum* produces 200 %, and *Rhizopogon roseolus* about 1600%.

11.5.3. Efficient visualization and analysis of fluorescently labelled subcellular structures of living yeast cells

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Two novel procedures and sample applications for efficient visualization and statistical analysis of fluorescently (multi)labelled subcellular structures in living yeast cells are presented. First, we show a threshold-based technique for semi-automatic registration of GFP-labelled, three-dimensional yeast structures. For this purpose, we take advantage of features of a modular software system (amira®), as well as of a novel highly efficient image reconstruction algorithm for counting yeast cells in differential interference contrast (DIC) images. Second, we introduce a custom-made software add-on for amira® visualization platforms, called '4D Assistant', which facilitates and accelerates the extraction of useful information from complex multi-channel 4D data sets. The tool was applied for simultaneous visualization of the migration of endoplasmic reticulum and mitochondria during cellular growth and division. Both techniques are not only highly useful for yeast specific applications but also applicable to other biological systems.

11.5.4. Three-dimensional visualization of fungal nuclei in binucleate ascospores

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Confocal laser scanning microscopy (CLSM) and 3D-visualization are techniques of choice for detection and three-dimensional visualization of complex fungal nuclear structures. In *Xanthoria parietina* asci, we studied the development of the eight ascospores which undergo an additional mitosis in order to become binucleate. Our study of the fungal nuclei comprises three steps: 1) We developed a method for specific and simultaneous staining of fungal nuclei in lichens with high contrast. The method includes NaOH-treatment of fixed sectioned samples and subsequent nuclear staining using SYTOX Green™. 2) We analysed these pretreated sections by use of a confocal laser scanning microscope. Both, high contrast of stained nuclei and fluorescence of cytoplasm provided advantageous preconditions for extraction of volume data using different 3D-visualization techniques. 3) Applying a state-of-the-art image processing software to the data from the stacks of pictures of the z-axis, we got good insight into the nuclear behaviour of the binucleate ascospores.

11.5.5. Microscopy and molecular biology join forces to unravel the mysteries of haustorial function

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Obligate biotrophic fungi such as the rust - and the powdery mildew fungi are important plant pathogens. The analysis of the underlying host-parasite interactions is faced with a variety of obstacles. For example, obligate biotrophic fungi cannot be genetically modified or grown without their respective host plants. This excludes them from the application of a variety of modern techniques successfully used on other pathogens. We are trying to elucidate the roles of one of the hallmarks of obligate biotrophic interactions, the so called haustoria. These are specially differentiated hyphae formed inside an infected plant cell. The

close contact between the haustorium and the host cell is an ideal prerequisite for the exchange not only of nutrients, but also of information. Our laboratory has established a combination of molecular and cytological tools to elucidate the processes involved in establishing and maintaining the obligate biotrophic state using the interactions of rust fungi with a variety of host plants as a model system.

11.5.6. In vivo observation of nuclear envelope removal during "open mitosis" in the basidiomycete plant-pathogen *Ustilago maydis*

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In animals and higher plants the nuclear envelope disassembles in mitosis to allow the interaction of chromosomes with the mitotic division machinery. Although lower eukaryotes are commonly thought to undergo mitosis within a closed envelope, ultrastructural data indicate that many basidiomycete fungi undergo an "open mitosis", including the pathogenic fungi *Cryptococcus neoformans* and *Ustilago maydis*. Here we describe the mechanism of nuclear-envelope breakdown in *U. maydis* using life cell imaging. In interphase the nucleus is located in the mother cell. Prior to mitosis, the spindle-pole body nucleates microtubules that reach into the daughter cell. Dynein at microtubule tips appears to exert force on the spindle-pole body, which leads to the formation of a long nuclear extension that reaches into the bud. Chromosomes migrate through this extension and, together with the spindle-pole bodies leave the old envelope, which remains in the mother cell until late telophase. Breakage and removal of the envelope required nuclear migration into the bud, as misplaced nuclei underwent a "closed" mitosis. Our data indicate that dynein-mediated pre-mitotic nuclear migration participates in nuclear envelope removal in *U. maydis*.

11.5.7. TEM-based studies for the occurrence and characterization of a mycovirus in sunflower downy mildew *Plasmopara halstedii*

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TEM studies using a negative staining technique revealed the presence of a virus in 23 of 26 isolates of *P. halstedii* deriving from 5 European and 3 American countries. Ultrathin sections showed the virus either dispersed throughout the cytoplasm or in membrane-bound vesicles in all stages of its host's life cycle. The virus occurred in 8 different pathotypes of *P. halstedii*, but was absent in sunflower and in 8 other species of Oomycota. The virus measured 37 nm in diameter. Its isometric capsid consisted of a polypeptide of 36 kD. Two major types of single-stranded RNA were detected comprising of 3.3×10^3 and 1.6×10^3 nucleotides, respectively. No significant differences neither in morphology nor in biochemical composition between the viruses of different *P. halstedii* isolates were found. The virus found in this study did not differ from the *P. halstedii*-Virus, first found in an isolate by Gulya et al. (1990) and described by Mayhew et al. (1992).

11.6.1. Phylogeny and evolutionary patterns in Nymphaeales: integrating genes, genomes and morphology

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The eight extant genera of Nymphaeales comprise species-poor (*Brasenia*, *Ondinea*) and species-rich (*Nymphaea*, *Nuphar*) genera and major lineages distributed on different continents. To evaluate monophyly and phylogenetic position of Nymphaeales genera, a dataset comprising multiple non-coding and fast-evolving regions from all three genomes was generated for a dense sampling (30 species). Three major lineages are resolved: *Nuphar*, Cabombaceae and core Nymphaeaceae (remaining genera). This dataset complements an enormous amount of phenotypic data, which is available through works of over a century. Based on molecular trees, the evolution of important characters such as floral architecture, reproductive systems, pollen morphology, and ploidy levels is reconstructed. Phenotypic data are evaluated for their support of insufficiently supported nodes of molecular trees based on combined data. The phenotypic data are placed in a

phylogenetic context in an effort to bridge the gap to the fossil record.

11.6.2. Nymphaeales - phylogeography and spatial diversification

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Nymphaeales, consisting of 70 species in 8 extant genera (*Barclaya*, *Brasenia*, *Cabomba*, *Euryale*, *Nuphar*, *Nymphaea*, *Ondinea*, *Victoria*), constitute the "first" clade of extant angiosperms with global distribution. Within the family biogeographic patterns differ strongly, ranging from local endemics (e.g., *Ondinea*) to cosmopolitans (e.g., *Brasenia*). Moreover, continental disjunctions of closely related taxa like *Victoria* (South America) and *Euryale* (East Asia) raise questions on the biogeographic history of the family. In this study we analyze extant patterns of biodiversity in Nymphaeales using a GIS database including all species. A multi-gene phylogeny with a dense taxon sampling designed to cover the geographically distinct lineages (including all subgenera of *Nymphaea*) serves to uncover historical processes and causes for recent distributional patterns. Ancestral areas are reconstructed by dispersal-viceariance analysis (DIVA). The findings are compared with the fossil record of Nymphaeales to evaluate whether recent distributions are rather relictual or are the outcome of recent radiations.

11.6.3. Pollination biology of night-flowering *Nymphaea* (Nymphaeaceae) species

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The large and specialized flowers of some Nymphaeaceae are exceptional among basal angiosperms. The genus *Nymphaea* is classified in five subgenera of which three are day-blooming and pollinated by bees and syrphid flies. Neotropical subgenus *Hydrocallis* is night-flowering with large dynastine beetles as pollinators. The specialized pollination mode is similar to the related genus *Victoria*. Although nocturnal like *Hydrocallis*, the flowers of the palaeotropical subgenus *Lotos* remain open until noon. *Nymphaea lotus* of the subgenus *Lotos* is pollinated by a dynastine beetle species as well as generalistic bees. It is likely that slower flower closure enables alternative pollinators to visit and pollinate flowers during the early day. The close relationship between *Hydrocallis* and *Lotos* regarding morphology and beetle pollination provide evidence that specialized beetle pollination was the original pollination mode of *N. lotus* and the spatial and temporal absence of the beetles are responsible for the observed generalized pollination. Future work will focus on floral traits selecting pollinators such as flower opening and scent emission.

11.6.4. Divergence times and historical biogeography of Nymphaeales

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Nymphaeales (Nymphaeaceae and Cabombaceae) comprise eight genera and approximately 70 species, with a worldwide distribution in tropical to temperate regions. Previous analyses of molecular and morphological data have provided a well-resolved and strongly supported generic-level phylogeny for the order. From published nuclear 18S rDNA, and plastid *rbcl* and *matK* DNA sequences, we estimated the divergence times of genera in Nymphaeales. We applied four different methods, a strict molecular clock, nonparametric rate smoothing, penalized likelihood, and a Bayesian method, to estimate divergence times. Our results indicate that extant Nymphaeales diversified into major clades during the Eocene (44.6 ± 7.9 mya); extant genera of Nymphaeaceae date to 41.1 ± 7.7 mya, and extant Cabombaceae diversified during the Miocene (19.9 ± 5.6 mya). Whereas the stem lineage of Nymphaeales is old based on fossil evidence (125-115

mya), our results indicate that extant Nymphaeales diversified relatively recently. We infer that the ancestor of Nymphaeales occupied the American and Eurasian continents during the Eocene; this inference is supported by the fossil record.

11.6.5. Lower Cretaceous evidence for the Cabombaceae water lily lineage and use to leaf architectural characters to identify major clades

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Molecular phylogenies suggest that there are three clades in the Nymphaeales. The *Braseria*-like fossils from the Albian of Jordan have orbicular, simple, entire margined, peltate, alternate leaves with long petioles attached to slender shoots. Venation is palmate with four (five) primary veins of which one has pinnate secondaries and the others branch dichotomously. Primaries and secondaries curve to form brochidodromous loops. The tertiaries are variable and intergrade with the quaternaries in a reticulate to random reticulate manner, although some leaves, like the stems and petioles, have little detail. The affinity to the Nymphaeales is based on the peltate attachment, leaf shape, leaf architectural characters, and the apparent aquatic nature of the leaves and stems covered with mucilage while to Cabombaceae clade is based on the shape of the leaf and the aquatic shoots, as opposed to rhizomes. Fossils show at least two clades existed by the Albian 110mya. An examination of leaf architecture characters reveals consistent characters to identify the order and show trends in the clades and may allow understanding of the detailed relationships of the fossils.

11.6.6. Environmental control of sepalness and petalness in waterlilies

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The conventional concept of an "undifferentiated perianth" obscures the fact that individual perianth organs may be differentiated into sepaloid (sepal like) and petaloid (petal like) regions. We have observed such differentiation in genera from several families including the basal angiosperms *Nuphar* and *Nymphaea* (Nymphaeaceae). In *Nuphar* the perianth organs traditionally called sepals exhibit both yellow petaloid and green sepaloid patches that show anatomical distinctions in addition to color differences. In *Nymphaea* the perianth organs near the boundary between "sepals" and "petals" are often subdivided into sepaloid and petaloid regions, rather than being intermediate in morphology over their entire surfaces. Our data suggest that the environment of a perianth organ helps specify these regions. The traditional concept of "sepal" and "petal" includes a hidden assumption that we believe is false: that sepalness and petalness must refer to whole perianth organs. We suggest a novel theory of perianth evolution, in which differentiation of the perianth into sepals and petals was predated by the evolution of sepalness and petalness.

11.6.7. Over 12,000 new *Nuphar* and *Amborella* gene sequences shed light on ancestral angiosperm transcriptome

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Multigene reconstructions of the seed plant phylogeny place *Amborella* and Nymphaeales as successive sister lineages to all other extant angiosperms or *Amborella* plus Nymphaeales as the basal clade. Genomic investigations of these lineages will, therefore, aid inference of gene content and genome structure in the most recent common ancestor of all extant angiosperms. The Floral Genome Project has generated over 17,000 expressed sequence tags (ESTs) for genes expressed in early flower development in *Amborella trichopoda* and *Nuphar advena*, representing over 6000 genes for each species. A concentration of duplicate genes identified in the *Nuphar* data suggests that it is an ancient polyploid. Phylogenetic analyses of gene families including sequences from *Amborella*, *Nuphar* and other angiosperms are elucidating the minimal set of floral development genes in the most recent common ancestor of all extant angiosperms and identifying potentially novel floral regulators. The new *Amborella* and *Nuphar* sequences provide critical references for estimating the timing of

gene and genome duplications relative to the earliest branching events in angiosperm history.

11.7.1. Gentians were not always blue - the why, when, and where of gentian evolution

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The gentian family has been the focus of intense phylogenetic research on tribal, generic and species level for over 10 years and we now have a new understanding of its complex historical patterns. Currently over 30 scientist are part of this on-going effort (see Gentian Research Network, <http://www.rci.rutgers.edu/~struwe/gentnet/>). This widespread family of 90+ genera and 1600 species contains a wide morphological, ecological, and distributional diversity. Recent analyses focusing on monophyletic groups of all levels and all geographic areas (Latin America, Indian Ocean Basin, etc.) utilizing GIS, molecular clocks, and molecular-based evolutionary hypothesis have lead to a deeper understanding of the evolution of this diversity. This overview provides a couple of peeks into the history of the originally-not-so-blue gentians and their tropical ancient lineages. For example, detailed species level studies in South America show environmentally-induced speciation in tropical forests and mountains. Recent results in all six tribes are reviewed with their associated insights in morphological, karyological, and biogeographic evolution.

11.7.2. Common weeds to rare habitat specialists: Speciation and distribution patterns of *Chelonanthus* within South America

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The neotropical genus *Chelonanthus* Gilg (Helieae) has been determined to be paraphyletic based on cladistic analyses of 5S-NTS and ITS sequences, and is composed of two lineages. One consists of *C. purpurascens*, with purple corollas and pollen shed as polyads. The second is all other species with green, yellow, to white corollas and pollen shed as tetrads. Molecular and detailed morphological characters are being analyzed to determine the relationships within and between the taxa. These species exhibit a continuum of habitat strategies, and another aim of the study is to investigate the evolution of habitat strategies within the light-colored corolla species. Within *Chelonanthus* examples of wide-ranging weeds, wide-spread habitat specialists and narrow endemics exist. Phylogenetic relationships and biogeography are analyzed to determine if habitat specialists are derived from habitat generalists and to elucidate the geographic origin in a historical perspective.

11.7.3. Tracing back the modes of speciation in *Halenia* (Gentianaceae) using a combined phylogenetic and GIS approach

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A molecular phylogenetic analysis of *Halenia* reveals clear cut large scale biogeographical relationships and the analysis of diversification rates shows that migration to new regions was followed by an immediate increase of speciation rates relative to sister taxa from regions which have been colonized earlier. To test whether speciation rates in *Halenia* are generally triggered by new possibilities of spatial isolation or by ecologically driven divergence we applied GIS (global information system) data on digitized distribution data of all species. The detailed distributions have become available as part of a complete taxonomical revision of the genus. We have developed macroecological niche models for each clade and used these to predict the distribution of sister clades. In some cases the predictions of the ecological niche model is excellent in others rather poor. The first result may point to allopatric speciation and the latter to ecological niche differentiation. Quantitatively the first process seems to have been more important in *Halenia* than the second.

11.7.4. Phylogenetic relationships within the genus *Sebaea* s.l. based on molecular and morphological data: evidence of polyphyly and its consequences on the evolutionary history of the tribe Exaceae.

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To reconstruct the evolutionary history of the genus *Sebaea* s.l. (ca. 85 species), molecular analyses were performed using 76 cpDNA (*trnL-F*) and 56 nrDNA (ITS) sequences. In addition morphological characters were included to a combined data matrix to detect possible non-molecular synapomorphies. Both molecular data sets highly support the monophyly of the tribe Exaceae, and further reveal the polyphyly of the main genus *Sebaea*. A first well-supported and basal clade comprises *Sebaea* s.s., a genus of mainly South African distribution. The remaining species form a more derived clade that consists of a primarily African genus - treated here as *Exochaenium* - that is closely related to *Exacum*, *Tachiadenus*, *Ornichia* and *Gentianothamnus*. Further, preliminary morphological data support a distinction between *Sebaea* and *Exochaenium*, whose mainly differ by floral characters. Finally, biogeographical patterns can be inferred for the tribe Exaceae, with a South African origin (including the Cape region) for *Sebaea*, followed by several migrations to Central Africa (*Exochaenium*), Madagascar (*Exacum*, *Tachiadenus*, *Ornichia*, *Gentianothamnus*), and Asia (*Exacum*).

11.7.5. *Prepusa* and *Senaea* (Helieae-Gentianaceae): endangered and endemic genera from Brazil with six-merous flowers

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Prepusa Mart. and *Senaea* Taub. (Gentianaceae) have never been revised since their original description in the 19th century. Based on analysis of herbarium collections from several herbaria, five species of *Prepusa* and two species of *Senaea* were recognized. A close relationship between the two genera is supported by sharing 6-merous flowers, a unique pollen tetrad type, and a membranaceous calyx. The 6-merous flower is a unique morphologic feature within the neotropical tribe Helieae, otherwise nearly exclusively 4- or 5-merous. *Prepusa* species present a large and showy calyx, longer than or almost as long as the corolla, whereas the *Senaea* species have a calyx much smaller than the corolla. These two gentian genera are rare montane herbs or shrubs endemic to restricted areas of four Brazilian states (Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro). A taxonomic revision of these two genera is presented including field keys to species, morphological descriptions and illustrations, current status, and maps on their geographic distribution to aid in the conservation of these endangered and evolutionary unique species.

11.7.6. Phylogenetic relationships within the New World endemic *Zeltnera* (Gentianaceae-Chironiinae) inferred from molecular and karyological data

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The New World endemic genus *Zeltnera* consists of 25 species mainly distributed in the western part of the United States and Mexico. Chromosome counts performed on 149 populations (24 species) reveal extensive congruence between chromosomal groups and the assemblages obtained from analyses of nuclear ribosomal DNA (ITS) and chloroplast DNA (*trnL* intron and *trnL-F* intergenic spacer) sequences. Karyological and molecular data sets support three main biogeographic groups for *Zeltnera*. A first and mainly unresolved cluster ($n = 17$ and $n = 20$) occurs in California, whereas two other clades are centered in the Texas region ($n = 20$ and $n = 21$) and Mexico ($n = 21$ and $n = 22$), respectively. Under the assumption of a molecular clock, and using both dispersal and vicariance explanations for the current distribution of the respective species, we can hypothesize a North American origin for the genus, with considerable diversification in the early Pliocene (ca. 5 mya). Geological events, such as desert formation and mountain orogenies, have created insuperable barriers that today separate the three major and likely vicariant groups.

11.7.7. Systematics of Macrocarpaea (Gentianaceae) including an infrageneric classification based on seed morphology, palynology, floral morphology and molecular characters

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Macrocarpaea has numerous seed and pollen types that have been systematically useful in establishing an infrageneric classification together with a phylogeny based on ITS and 5s-NTS sequences. The genus of 106 species has an origin in the Atlantic coastal forest of Brazil, yet has undergone large-scale speciation within the Andean Cordillera. Four sections are recognized within Macrocarpaea: Sect. *Tabacifoliae* comprises 4 species that occur in Southeastern Brazil and characterized by Flattened-type seeds. Sect. *Macrocarpaea* comprises 42 species largely of the Northern Andes (Colombia and Venezuela), southern Mesoamerica (Costa Rica and Panama), Pantepui of the Guayana Shield, and the Greater Antilles of the Caribbean (Cuba, Hispaniola, and Jamaica) characterized by Rimmed-type seeds. Sect. *Magnifoliae* comprises 18 species of the Central Andes (Ecuador, Peru, and Bolivia) characterized by Corymbosa-type Pollen and Winged-type seeds. Sect. *Choriophylla* comprises 37 taxa largely of the Central Andes (W Colombia, Ecuador, Peru, Bolivia), especially the Huancabamba region of Ecuador and Peru characterized by Perimetrically winged-type seeds.

11.8.1. Understanding the systematics, ecology and evolution of Podostemaceae: What is the future?

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This talk will summarize recent advances and highlight key areas that remain poorly known in the understanding of Podostemaceae. Taxonomic insight has improved dramatically in some geographic regions but remains inadequate in others where taxonomic circumscriptions remain suspect. The incidence of monotypic genera at ca. 40% illustrates the present artificial genus concept. Exciting insights have come from the application of phylogenetic principles to probe questions of evolutionary relationships, biogeographic patterns, and character evolution, yet these have been applied to a minority of the family. Understanding of ecology remains poor. Factors that influence local species distributions remain unknown and the role that plants play in the ecology of tropical rivers is little clarified. Essentially nothing is known about how genetic variation is partitioned within and between populations. Such insight would be valuable for addressing taxonomic, ecological and conservation issues. Conservation concerns remain to be clarified. Reported high species endemism, combined with intense human impacts on their habitats, are a sobering combination.

11.8.2. Molecular phylogeny and biogeography of Podostemaceae

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The matK-deduced phylogeny of Podostemaceae shows that the family is divided into three subfamilies. Podostemoideae has several clades comprising the largest number of species. The basalmost is an American clade, and the rest of the subfamily is divided into an Afro-American clade and an Asian clade. Tristichoideae is a small, basically Asian subfamily with an exception of Afro-American *Tristicha*, the ancestor of which likely migrated from Asia. Weddellinoideae is a monogeneric American subfamily being sister to Podostemoideae. The phylogenetic pattern may reflect the biogeographical history of major groups. It is likely that Podostemaceae underwent an early geographic split to Asia (as *Tristichoideae*) and America (as *Weddellinoideae* + *Podostemoideae*), and then parts of *Podostemoideae* expanded to Africa or migrated to Asia. The geographic changes accompanied remarkable modifications in embryonic developmental pattern and the resulting body plan. *Dalzellia* and *Indotristicha* (*Tristichoideae*) and *Cladopus* and *Hydrobryum* (*Podostemoideae*) exhibit saltational evolution.

11.8.3. Systematics, phylogeny, and conservation of podostemads in the New World.

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The taxonomy of Podostemaceae in the Americas started in 1775 with the description of *Mourera fluvialilis* from French Guyana. Subsequently nearly 160 species (in 19 genera) have been described. Though Peter van Royen (1950s) published the most comprehensive taxonomic treatment of New World Podostemaceae, much of the taxonomy remains tentative. The circumscription of many New World genera is troublesome. The genus *Podostemum* will be used to illustrate the use of phylogenetic analyses to redefine generic boundaries. The results of phylogenetic analyses will also demonstrate difficulties involved in the circumscriptions of such genera as *Apinagia*, *Marathrum* and *Oserya*. The emphasis on hydroelectric power (dam building) in South America, combined with reported high levels of species endemism, leads to conservation concerns in the family. Yet such concerns can be difficult to clarify because of taxonomic uncertainty. The genus *Castelnavia* will be used exemplify this problem. Finally, the importance of field collecting will be shown by discussing taxonomic diversity of Podostemaceae in Mexico and southern Brazil (especially *Podostemum*).

11.8.4. Systematics, phylogeny and conservation of Podostemaceae in Africa

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The first accounts of the Podostemaceae from Africa appeared in the literature in 1849 and 1852 by Tulasne. Since then about 80 publications on African (including Madagascar) Podostemaceae are available. Africa has 16 genera and c. 79 species, making it the second center of biodiversity, apart from tropical America. Eight out of the 16 genera are monotypic and occur in Africa and Madagascar; five of them have monotypic genera restricted to Africa; two of them are monotypic genera endemic to Madagascar; and one species is widespread in Africa and Madagascar. Seven other genera consist of 2 - 7 species. The genus *Ledermannia* is exceptionally large, containing c. 45 spp. It contains more than half of all Podostemaceae taxa from Africa. Most Podostemaceae genera and species in Africa occur in the mountains of Cameroon and Gabon. Several African taxa are not intensively studied. The Ghanaian Podostemaceae, with c. 4 endemic species and a widespread one, however, are well known due to intensive field work and additional lab studies.

11.8.5. Embryological and reproductive structures of Podostemaceae

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Flowering is at the intersection of submerged to aerial life at subsiding water level when the plants become exposed. It means a change from aquatic to xerophytic life on sun heated rocks. This condition necessitates sure and rapid fertilisation (2-3 d) and resources present for a period of gradual limitation of supply at seed ripening (2-3 w). The strategy of Podostemaceae to accomplish these needs is manifold: Fertile pollen and fertilisable embryo sacs developed at submerged life; pollination autogamous or cleistogamous, rarely allogamous; acceleration of the embryo sac development (restriction to the micropylar quartet of the embryo sac, reduced *Allium* type); no double fertilisation, hence no endosperm; but already existing tissue as a nutrient of the embryo ("nucellar plasmodium"); suspensor haustorium; the outer cells of the testa mucilaginous upon wetted; high seed set, but largely propagated by clonal growth. The particularities are discussed, new own results on microsporogenesis and new palynological data presented.

11.8.6. Organ identity of the plant body in some Indian Podostemoideae (Podostemaceae)

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The Podostemaceae is a unique family of aquatic angiosperms. The Indian Podostemoideae are characterized by a dorsiventrally thaloid plant body. The nature of the plant body has long been a subject of dispute. Determining the origin, structure and function of an organ can give an insight into its identity. We have traced the ontogenetic details from seed germination to seedling establishment and studied the structure and development of the plant body in a few Indian podostemads to interpret the thallus and its components. The cytohistological zonation of the thallus tips in

these taxa is similar to the tunica- corpus of the shoot apical meristems of typical angiosperms. The secondary vegetative shoots that arise endogenously prior to the initiation of lateral thallus branches, are stemless and show limited growth. The lateral thallus branches arise exogenously and are proximal to secondary vegetative shoots. They repeat the same growth pattern as that of the main dorsiventral stem. The lateral thallus branch can be interpreted as a structure with fuzzy boundaries.

11.8.7. Comparative and developmental morphology of roots and shoots in African Podostemaceae.

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Podostemaceae show morphological novelties not known from other flowering plants, e.g. crustaceous or ribbon-like green roots (many taxa), terminal double-sheathed leaves (e.g. *L. bowlingii*, Ghana), endogenous formation of flowers along stems due to dedifferentiation of cortex tissue (e.g. *Ledermannia letouzeyi*, Cameroon), epiphyllous flowers (e.g. *L. prasina*, Cameroon). Several unique morphological characters of African Podostemaceae are listed in the homepage www.systbot.unizh.ch/podostemaceae/. Some architectural peculiarities of African Podostemaceae (e.g. double-sheathed leaves) are also known from American and Asian taxa. Botanists disagree about how to best interpret the vegetative body of Podostemaceae. It may be described as partially homologous with the classical root-shoot model that applies to most other angiosperms. Structural terms such as 'root', 'shoot', 'stem' and 'leaf' in Podostemaceae do not necessarily imply a total homology with these categories in typical angiosperms.

11.9.1. The Proteaceae: divergences, dates and classification

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Following the analyses of several DNA sequence data sets, the classification of the Proteaceae requires re-alignment. We present a new classification at subfamily, tribal and subtribal rank. Five subfamilies, 12 tribes (two of which are new) and 11 subtribes (three of which are new) are recognized. However, numerous taxa are considered *incertae sedis*, and await further examination. Using a subset of taxa representing major lineages of the family, the molecular data (*atpB*, *rbcl* and the *atpB-rbcl* spacer regions) were subjected to various molecular dating analyses using several fossil calibration points. Our results for some clades are consistent with the widely held hypothesis that the Proteaceae are a "Gondwanic group", with trans-oceanic disjunctions caused by continental drift. However, some clades with intercontinental distributions are estimated to be much younger than the ocean basins that they span. These anomalies are striking enough to challenge biogeographic orthodoxy.

11.9.2. The fossil record of fruits of the Proteaceae

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The record of fossil fruits of the Proteaceae is reviewed based on comparative studies involving morphological and anatomical characters with fruits of extant members of the family. Fossil fruits/infructescences that unambiguously belong to the family are confirmed for the Tertiary of Australia and South America. Fossil fruits reported from the Tertiary of New Zealand and Antarctica may also belong to the family, but require further investigation.

The majority of fruit taxa are consistent with fruits of subfamily Grevilleoideae, a notable exception being the large distinctive fruits, recovered from mid-Tertiary sediments in southeastern Australia, of *Xylocaryon lockii* which conform with those of *Eidothea*, currently nested within the subfamily Proteoideae based on chloroplast DNA sequence data. The record of subfamily Grevilleoideae includes *Orites* follicles from the Paleocene of Patagonia, "cones" of *Banksia* from the Paleogene and Neogene of Australia, and indehiscent to tardily dehiscent fruits from latest Eocene-Miocene sediments of eastern Australia allied to those of several taxa within tribes Macadamieae and Grevilleeae.

11.9.3. A phylogenetic approach to the fossil record of Proteaceae (Proteales): insights from the Banksieae

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Proteaceae (1700 spp) have a particularly rich fossil history throughout southern Gondwana. Extending back to the mid-Cretaceous they thus represent an ideal model to test biogeographic hypotheses in the Southern Hemisphere. However, the appropriate use of fossil data in Proteaceae has so far suffered both from methodological problems and insufficient knowledge of morphological diversity within the extant family. Here we use tribe Banksieae (180 spp) to illustrate the need for a more rigorous approach to fossil Proteaceae. Based on special features of this group, various palynomorphs and macrofossils from the Cenozoic of Australia and New Zealand have been assigned to Banksieae without ambiguity. In particular, diporate pollen grains are almost exclusive to the four genera in the tribe. We report new substantial palynological data on extant Banksieae, and discuss the assignment of diporate palynomorphs and serrate leaves and their reliability as calibration points in molecular dating analyses.

11.9.4. Morphology, genes, and fossils to infer evolution in Platanaceae

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The family Platanaceae consists of a single genus today that comprises only 7 species. The fossil record shows that the family extends back to the mid Cretaceous, and, based on findings of reproductive structures and leaf fossils, suggests a much higher diversity of the family for the Cretaceous and early Cenozoic. A combined morphological-molecular study shows a) three distinct genetic lineages within *Platanus*: The East Asian *P. kerrii*, an eastern N American/Central American clade, and a Pacific N American-European clade, b) that all three lineages appear to be present in the fossil record already in the Cretaceous, c) that a number of morphological characters are stable from the Cretaceous to present (pollen), d) that leaf epidermal features in modern *Platanus* are more complex than previously assumed but basically do follow a general pattern found in basal angiosperms and basal eudicots, which limits its value to discriminate fossil "Platanaceae" against other families, e) that leaf architecture provides a valuable tool to discriminate *Platanus* against other taxa, and, finally, that no indication for a close Proteaceae-Platanaceae relationship exists.

11.9.5. Cuticular features of fossil Platanaceae and their taxonomical importance

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Platanoid foliage and reproductive structures are known from middle Albian. The taxonomical and morphological diversity of extinct Platanaceae in the Cretaceous and Palaeogene was significantly higher than diversity of extant family. All representatives of the family Platanaceae possess very similar and rather specific cuticular structure. The investigation of intact cuticle of fossil leaves allows the recognition of platanaceous affinity of many morphotypes, because their systematic position is not always obvious from morphology. Recently about 15 genera of fossil platanoids have been distinguished on the basis of leaf morphology and cuticular structure. The diversity of epidermal characters of the Cretaceous platanoids was also higher than is known for modern species of *Platanus*. Some extinct genera are characterized by a diagnostic complex of specialized cuticular features. The investigation of cuticle structure also provides a means to distinguish species with similar morphology, because morphological variability of the majority of taxa is very high.

11.9.6. Fossil reproductive structures: re-evaluation of Cretaceous diversity in Platanaceae

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Extant members of the families Platanaceae and Hamamelidaceae are easily differentiated by their reproductive morphology. The recent *Platanus* is characterized by naked flowers having an apocarpous gynoecium with a varying number of elements. In contrast, extant Hamamelidaceae are characterized by a syncarpous semiinferior ovary of two carpels. In the fossil record platanoids and hamamelids (in particular altingioids) are often similar in the macromorphology of their reproductive organs making their distinction difficult. For example, the extinct platanoids had a constant number of carpels per flower and a well-developed perianth. Among the hamamelids extinct types with monocarpellate flowers are known. Although extant altingioids have naked flowers, our study has revealed well-developed perianths in early hamamelids as well. These findings suggest the previously believed high Cretaceous diversity of Platanaceae needs re-evaluation. This work is supported by the Russian Foundation for Basic Research, project 03-05-64794.

11.10.1. Grapevine imprinted by natural and human factors

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I Genetic inheritance of grapevines involved with vegetative multiplication.

- Factors enhancing diversity.

- Typicity and its constraints including the limits of varietal creation and considering the effects of viruses.

Conclusion regarding the variable nature of vegetative multiplication focusing on variability outside of sexual breeding.

II Phenotypic character of grapevines depending on natural factors.

- Environmental factors such as soil, water stress, solar orientation, climate....

- Biological factors. Examples concerning natural defences will be detailed. Elicitation with resveratrol response will be discussed looking at the process from the genus to the leaves, to the berries and the wine. External effects of resveratrol existence on fungi and human consumers will be shown. Looking at these new factors as material for continued evolution.

Conclusion : the preexisting notion of "terroir" and its relationship with the grapevine characteristics detailed in this discussion.

11.10.2. Shoot architecture in the Vitaceae

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Most Vitaceae possess a distinctive shoot architectural pattern, characterized by leaf-opposed tendrils. Shoots are modular, consisting of three-node repeating cycles. Five architectural models have been described, based on the pattern of tendril and axillary bud occurrence at each node. The relationship between mature growth form, shoot architecture, and shoot ontogeny will be compared in three species of *Cyphostemma* (*C. juttae*, a herbaceous succulent with terminal inflorescences, lacking tendrils, *C. mappia*, a woody shrub with terminal inflorescences, lacking tendrils, and *C. simulans*, a liane, with leaf-opposed tendrils and axillary inflorescences). These results will be discussed in the context of the relationship between mature architecture and shoot ontogeny. Evidence for the most likely evolutionary pathway, from Pattern 1 with spiral phyllotaxy and no tendrils, to Pattern 5 with distichous phyllotaxy and continuous tendrils and axillary buds, will be presented. The question of whether this unique shoot pattern should be considered to be monopodial, sympodial, or a combination will be discussed.

11.10.3. Molecular analysis of flowering transition in grapevine (*Vitis vinifera* L.)

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Flowering in grapevine (*Vitis vinifera* L.) seems to be promoted by the summer rise in temperature and light intensity. As a result, lateral meristems produced at shoot apical meristems in summer latent buds proliferate and differentiate to produce a cluster of inflorescence meristems in place of tendril primordia. After winter dormancy, inflorescence meristems will give rise to clusters of flower meristems and flowers. To understand the molecular mechanisms underlying this process, we have characterized *VFL*, *VAP1* and *VFUL*, putative *Vitis* orthologs of *LFY*, *AP1* and *FUL* genes, responsible for flower meristem identity in Arabidopsis. *VFL*

expression is detected in all analyzed meristems and primordia. However, consistent with a role in flower initiation, *VFL* transcripts accumulate in proliferating lateral meristems giving rise to inflorescence meristems. *VFUL* and *VAP1* are also highly expressed in lateral meristems and inflorescences and flower meristems. Interestingly, they also accumulate throughout tendril development. This expression pattern provides molecular support to the origin of tendrils as modified reproductive organs adapted to climb.

11.10.4. Floral development in the Vitaceae

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The common perception in the Vitaceae is that variation in reproductive structures is small and therefore of little use systematically below the generic level. This perception, unfortunately, is based on a small database of floral studies in the Vitaceae, especially very few floral ontogenetic studies. In the past twenty years we have examined the floral development of 12 species in 6 genera and have noted a significant amount of variation and a clear evolutionary trend. Floral characteristics shared in the Vitaceae include; inflorescence branching as compound dichasia, bisexual flowers, precocious initiation of a calyx ring, unfused non-showy petals which overarch the petal-oppoed stamens and bicarpellate gynoecia (four ovules) with septa forming from the inner ovular walls. In derived members of the Vitaceae (i.e. *Vitis*) certain floral structures such as the calyx ring and the nectary disk have been modified. The questions concerning an evolutionary trend in the flowers of the Vitaceae will be further explored by examining three members of the genus *Cyphostemma*, *C. juttae*, *C. mappia* and *C. simulans*.

11.10.5. On the diversity and evolutionary diversification of Vitaceae in Asia.

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Asia is one of the major centers of diversity for the grape family Vitaceae. Twelve of the 14 genera in the family are distributed in tropical and temperate Asia including three endemic genera: *Acareosperma*, *Nothocissus* and *Pterisanthes*. Several large genera such as *Ampelocissus*, *Tetrastigma* and *Vitis* have their highest diversity in Asia. A few genera have conspicuously disjunctive distribution between Asia and the New World. Our chloroplast sequence data (*trnL-trnF*, *atpB-rbcL* and *rps16* intron) suggest that the Asian species of *Cayratia* and *Tetrastigma* and the largely African *Cyphostemma* form a clade, with *Cayratia* possibly being paraphyletic. *Nothocissus* and *Pterisanthes*, two small Asian endemic genera, are nested in the clade of *Ampelocissus*. The position of *Yua* is not well resolved. The North American species of *Parthenocissus* are highly distinct from the Asian relatives. The results suggest that: (1) multiple migration between Asia and the New World; (2) ancient origins of the intercontinental disjunctions between the two continents in Vitaceae; and (3) the evolutionary radiation at both species and generic levels in eastern and southeastern Asia.

11.10.6. Vitaceae systematics in South America

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To date, in South America, the Vitaceae is represented by 65 species in 2 genera, *Cissus*, with 64, and *Vitis*, with one.

A recent study on the Neotropical *Cissus* accounted for 75 species, 62 of which in South America, but, these last two years, I have described two more.

My study reveals that, for species delimitation hair types and fruits and seeds morphology are more important than density of hairs, and leaf shape.

The traditional species groups are first delimited through leaf shape, a tricky characteristic for species determination because of the great variation of leaf shape and composition in the same species as well as in a one individual, since composite leaves have probably evolved many times in *Cissus*. Thirteen are recognized, excluding seven uncertain species.

These groups are provisional, and other characteristics such as anatomical and histochemical ones (latex has been observed in the *C. trigona* group) and molecular approaches should be performed to analyze a lot of useful data, contributing to a more

solid subgeneric division of *Cissus*, not only in South America but worldwide, with the collaboration of all taxonomists interested in this fascinating family.

11.10.7. Is the genus *Cissus* (Vitaceae) monophyletic? A molecular study of the Australian radiation

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The *trnL* (UAA) intron and the ITS1 region were sequenced to investigate relationships among the five genera of Vitaceae present in Australia. Mostly congruent results were obtained between separate and combined data sets, with all major clades being shared among trees. All bootstrap consensus trees obtained from single sequences or combined analysis strongly suggest that *Cissus* is polyphyletic, corroborating the morphological inconsistencies reported previously. *Cissus opaca* and *Clematicissus angustissima* consistently group in a clade sister to *C. tweediana* and *Rhoicissus tridentata*. A further four taxa (*C. antarctica*, *C. hypoglauca*, *C. oblonga*, and *C. sterculifolia*) also grouped within a clade clearly disjunct from the main *Cissus* group. Our results suggest that these five species currently classified as *Cissus* should be segregated from the genus. Of further interest is the close relationship between *Cayratia* / *Tetrastigma* and the lack of support for *Nothocissus* as a separate genus. Overall, the results presented provide new insights into the relationships within a number of Vitaceae genera and suggest directions for future studies.

11.11.1. Comparative population viability and life history of Florida scrub species and implications for fire management and conservation

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I integrate information from long-term studies, chronosequences, and experiments into life history and population viability analyses (PVA) of Florida scrub plants, to suggest evolutionarily relevant fire regimes (ERFR). Resprouting shrubs dominate more frequently burned scrub (scrubby flatwoods, hickory scrub) and will probably be resilient to a range of fire return intervals (FRI). For seeders, time to first reproduction, inter-fire demography of adults, and seed banks may constrain FRI. Florida rosemary scrub is dominated by a seeder (*Ceratiola ericoides*), which can be eliminated by frequent fires (< 10 years) or by fire suppression (> 80 years). Because we cannot yet closely specify ERFR based on life history information, PVAs provide more precise recommendations. For two species that grow in oak-hickory scrub, PVA suggests short (6-12 year) FRI. PVAs on two rosemary scrub herbs (*Hypericum cumulicola* and *Eryngium cuneifolium*) suggest frequent fires would promote their persistence. I argue that a moderate amount of pyrodiversity can promote biodiversity while not harming individual species' viability.

11.11.2. Demography of *Oritrophium peruvianum* and impacts of harvesting on its populations in the Venezuelan Andes

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The population dynamics of the medicinal plant *Oritrophium peruvianum* were analyzed using a five stage time-invariant linear matrix model. The growth rates of two populations monitored in the Venezuelan Andes were greater than one (1.32 and 1.13). Growth rates were more sensitive to changes in the survival of individuals than in reproduction. The most significant transition was the survival of small adults whilst large adults contributed little to their growth rates. Different harvesting regimes were simulated by removing different proportions of individuals from selected stage classes. A uniform harvesting of the adult stages each year up to 20-40% could be applied without threatening the population survivals. The harvesting of small adults would affect the growth rates of the populations most, whilst the complete removal of large adults would have the least impact. The optimal sustainable harvesting was calculated by maximising the total yield among different sustainable harvesting policies. The most sustainable practical policy would allow harvesting of all large adults and different proportions of the small and middle-sized plants each year.

11.11.3. Population stage structure as a quick-and-dirty population viability indicator: how dirty is it ?

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In the 1980's Rabotnov published a series of papers on 'coenopopulation structures' of plants. I adopted his way of looking at populations as a method to obtain a relatively good idea of the demographic viability of populations of threatened plants within one censusing season. The basic idea is to examine the stage structure of many (sub)populations and relate them to various aspects of habitat quality, such as vegetation composition and structure and soil variables, and different types of habitat management. For our study species, *Gentiana pneumonanthe*, *Salvia pratensis*, *Veronica austriaca* and *Liparis loeselii*, the method has proven very valuable for decisions concerning optimal habitat management, although its limitations also became apparent. In my paper, I will bring together our studies with those published on other species, to investigate whether this quick-and-dirty method of assessing population viability really works and to report on its pros and cons for the practical conservation management of endangered plant species.

11.11.4. Demography of the declining grassland plant *Trifolium montanum* in central Germany: the influence of site productivity

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We studied the demography of the long-lived, declining plant *T. montanum* and the structure of its populations in central Germany. Intensive demographic monitoring in nine populations revealed that with increasing nutrient availability at a site the finite growth rate of populations (λ) decreased. The decrease in λ was the result of reduced growth and increased mortality of non-reproductive plants. In a clipping experiment λ increased significantly from 0.91 in unmanaged to 1.13 in managed plots, indicating that the detrimental effects of high nutrient availability were due to increased light competition. The removal of 25% of the vegetation was enough to release light competition and to considerably reduce the mortality of non-reproductive plants. Nutrient availability also influenced the structure of populations. The density of immature plants strongly decreased with increasing productivity in 20 populations of *T. montanum*, indicating reduced recruitment at high productivity sites. Our results suggest that nutrient enrichment is the major cause of the decline of *T. montanum* and that many of the remnant populations are strongly threatened.

11.11.5. On the relationship between longevity and population dynamics: the demographic component of life history evolution in plants

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Theory predicted the evolution of life histories towards short or long lived life spans through life history trade-offs. The goal of this paper is to reveal that life span and demographic behavior are intricately related, emphasizing the demographic component of life history evolution. To this end, we investigated the relationship between longevity and population growth rate, a surrogate of global fitness. Our study included 24 demographic datasets of herbs using population matrix models. For each dataset of study, we computed longevity, mean and temporal variance in population growth rate, and demographic importance of life history traits (based on elasticity and variance contribution analyses). Results clearly indicate that long-lived plants exhibit more stable and less variable population behavior than short-lived plants. The relative importance of fecundity decreased with increasing longevity whereas that of survivorship showed the opposite pattern. We conclude that longevities are a good predictor of population dynamical trends in plants. Results are presented and discussed on the basis of the evolution of plant life histories.

11.11.6. Competitive effect is a linear function of neighbour biomass in *Kochia scoparia*

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To investigate the effects of neighbour size on competitive effect, we grew target *Kochia scoparia* individuals surrounded six equidistant, even-aged, conspecific neighbours. We varied neighbour size by sowing neighbours at different times, and we varied the sowing time for the target plants to generate variation in both neighbour and subject size. We analyzed the growth of focal plants over two time intervals as a function of their own size and the size of their neighbours at the beginning of the interval.

When competition was intense, the relative growth rate of target plants was primarily determined by the size of their neighbours. There was a negative linear relationship between the relative growth rate of target plants over and the biomass of their neighbours at the beginning of the interval. The size of the target plant itself did not make a significant additional contribution to predicating its growth rate.

There was no evidence of size-asymmetric competition. Neighbours larger than the target plant had more of an effect than smaller neighbours, but the per unit effect of neighbour biomass was the same for neighbours larger and those smaller than the target plant.

11.11.7. Effects of multiple herbivores on the population dynamics of a perennial herb

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We present a modelling approach to be used in estimating simultaneously the long-term fitness effects of multiple herbivores on perennial plants. We examined the effects of two specialist herbivores, a pre-dispersal seed predator and a leaf-feeding moth, on the population dynamics and long-term fitness of their shared host plant. We compared the relative roles of seed predation and leaf herbivory in determining plant population dynamics. Demographic data were collected during three years and this data was combined with the effects of natural levels of herbivory. Both seed predation and leaf herbivory reduced population growth of their host plant, but only very high damage levels changed the growth trend of the vigorously growing populations from positive to negative. Seed predation was more important in determining plant population growth than leaf herbivory. Moreover, the impact of leaf herbivory was insignificant in a situation where seed predation intensity was very high.

11.12.1. Leaf canopy as a dynamic system: ecophysiology and optimality in leaf turnover

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Leaf canopy is a dynamic system, where leaves are produced and fall with time. We developed a model of leaf turnover in a canopy (Hikosaka 2003 Am Nat). In the model, the rate of leaf production is a function of the canopy photosynthetic rate and leaf loss results from the leaf area that exceeds the optimal leaf area index. The model predicted environmental response of leaf area index and canopy photosynthesis. We further developed a model in which an individual has two canopies (e.g. two branches on a tree). When the two canopies are exposed to different light environment from each other, development of the two canopies depended on the degree of physiological integration between the canopies (i.e. reallocation of photosynthates and nitrogen). Using monospecific stands of an annual, we analysed leaf turnover and its environmental dependence. Both light environment in the canopy and nitrogen availability were shown to affect leaf production and shedding (Oikawa et al. 2005 Oecol).

11.12.2. Nitrogen-use efficiency in canopy and understory species in a beech forest

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We studied nitrogen (N) use in coexisting species in a mature beech forest. Leaf-level nitrogen use efficiency (NUE_L), defined as the amount of leaf produced per unit N taken up into leaves, was

similar between canopy (*Fagus crenata*) and understory (*Lindera umbellata* and *Magnolia salicifolia*) species. NUE_L was analyzed further as the product of the leaf-level N productivity (NP_L ; growth rate per unit leaf N) and the mean residence time of leaf N (MRT_L). Plants with higher NP_L can produce biomass more rapidly, while those with longer MRT_L can use the same N for a longer period. NP_L was significantly higher in the canopy species owing to higher irradiance. MRT_L , on the other hand, was significantly shorter in the canopy species mainly because wind removed a larger portion of green leaf before leaf N could be salvaged during autumnal senescence. We conclude that the canopy and understory species had similar NUE_L but through different mechanisms. This study also highlighted the importance of environmental incidents (e.g. wind) in determining plant performances in natural stands.

11.12.3. Foliage traits that determine the efficiency of light capture in mountain birch

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Structure, size and number of leaves per shoot can be variable within plant species. These traits participate in light capture together with the spatial arrangement of shoots. The amount of variation in leaf traits and 3D plant architecture in a mountain birch (*Betula pubescens* ssp. *czerepanovii*) population was measured and utilized in computer simulations to identify leaf traits that determine the efficiency of light capture and photosynthesis. Photosynthetic nitrogen use efficiency (PNUE) was estimated as the potential rate of photosynthesis (P_{max}) per nitrogen lost at leaf senescence. Shoot-specific PNUE followed an U-shape curve with respect to leaf weight per area ratio (LWA) that estimated nitrogen allocation. Small leaf area per shoot and low LWA was an efficient combination, and also observed in real trees. Small leaves and high LWA was another efficient combination, but not observed. High LWA was associated with large leaves of old stems where the nitrogen cost of leaf production increased without an adequate return in P_{max} . This resulted in decrease of PNUE despite higher P_{max} . Especially young mountain birch trees are sparing in their use of nitrogen.

11.12.4. Modeling light capture and carbon gain in tropical shrub seedlings: correlations with growth in different light environments

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Realistic 3-D reconstruction of plant crowns has led to accurate predictions of light capture and carbon gain, but explorations of how these predictions relate to plant performance remain scant. Seedlings of 15 *Psychotria* species that co-occur in a lowland tropical forest in Panama were grown in low, intermediate and high light. 3-5 plants per treatment and species were measured 4-8 times throughout their development during their first 1-1.5 year. Crown architecture was reconstructed with the computer model Yplant, leaf photosynthesis was measured following standard protocols and above ground relative growth rate (aRGR) was calculated from sequential biomass estimates. Light absorption efficiency was inversely related to self-shading and directly related to whole plant daily carbon gain (DCG). DCG as estimated by Yplant was a good predictor of aRGR in the intermediate and low light environments, but not in the high light environment where stress effects overrode any significant relationship. Gap and understory species overlapped in most measures though there was a tendency for gap plants to segregate out to higher carbon gains and aRGR.

11.12.5. Leaf and shoot biomechanics and light interception efficiency in temperate trees

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Intercepted irradiance per unit dry mass (light harvesting efficiency) widely differs among the leaves and shoots of varying architecture. This partly reflects the inherent trade offs between the high investment of photosynthetic resources per unit leaf or shoot surface area, and enhanced exposure of chlorophyll within the leaves and leaves within the shoots. While the first strategy improves the whole leaf and shoot photosynthetic potentials at high light, the second alleviates light limitations in low irradiance. Apart from the adaptational variation in light harvesting efficiency

with the total amount of light, biomechanical characteristics of foliage significantly alter the light harvesting efficiency of the foliage. In particular, leaves of varying size and shape require different fractional biomass investments in self-support to avoid self-shading and keeping leaves in positions maximizing irradiance capture. Apart from penumbra, variations in leaf size and shape are thought to affect plant light harvesting only to a minor extent. Due to different biomechanical costs, these species-specific features strongly alter light capture efficiency.

11.12.6. Biomass allocation and light capture in a very successional tropical forest stand

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We analyzed a 1 y/o secondary forest stand that contained a number of short- and long-lived pioneer species. It was hypothesized that at this stage, where plants from the two groups did not differ much in size, short-lived pioneers use their biomass more efficiently to grow tall and capture light giving them a competitive advantage. With a canopy model we evaluated interspecific differences in stem characteristics, leaf display and biomass allocation in terms whole plant light capture. Short-lived pioneers were indeed somewhat taller for a given amount of mass than long-lived pioneers. This was because they invested relatively more mass in their stem, and because they had lighter wood. Short-lived pioneers had lower leaf mass- and leaf area ratios. They captured more light in absolute terms and per unit leaf area or leaf mass. However they did not capture more light per unit total mass, which contradicted our hypothesis. Physiological characteristics enabling a high light-use efficiency might be more important in giving short-lived pioneers a competitive advantage than morphological traits that allow for a high light capture per unit mass.

11.12.7. Towards a mechanistic explanation of a steady state in growing trees

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When a tree ceases to grow in height, it continues to grow in stem diameter and may reproduce for decennia or even centuries. These observations suggest that adult trees have a constant amount of living biomass, and 'simply' replace leaves, flowers and fruits in the crown, and sapwood in the stem. What mechanisms contribute to the switch from vegetative expansion to reproductive steady state? And why do different tree species reach such a steady state at different heights and in different light environments? Using a new functional-structural plant growth model, we first show how the effects of tree size and shading (by surrounding forest and tree-crown) on the carbon economy and meristem responses result in a growing 3D-structure such as a tree. In reply to our questions, we show how inter-specific differences in meristem responses and leaf and sapwood turnover rates contribute to a steady state in tree species that grow to different adult sizes, and come to a steady state in different forest light environments.

11.13.1. Solar UV-B radiation, plants and terrestrial ecosystems

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Stratospheric ozone reduction has been the impetus for understanding how solar UV-B (280-320 nm) radiation affects plants from gene regulation to ecosystem-level ramifications. Depending on the environmental history of the plant and wavelength composition, solar UV-B can be damaging. But, usually repair and mitigation are effective in curtailing appreciable inhibition. Solar UV-B can also regulate many plant processes such as altered growth allocation and secondary chemistry. These, in turn, can be manifested at different tropic levels such as altered herbivory, decomposition of plant litter, and plant competition. Changes in soil microbial activity, peatland microfaunal and fungal biodiversity have also been attributed to UV-B effects mediated through the higher plants. Direct UV-B effects on fungal communities exposed to sunlight on litter and leaf surfaces also have been reported. Finally, some plant and ecosystem effects appear to be the result of abiotic UV-B photochemical effects on

litter and the lower atmosphere. In order for all of these effects to be relevant to ozone layer reduction, the UV responses must have specific spectral properties.

11.13.2. UV-B induced responses of plants at the molecular level: What we know and what we need to know

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Molecular events underlying plant responses to UV-B radiation are beginning to be understood. UV-B clearly impacts at the level of the transcriptome. This modification of gene activity is not merely a consequence of DNA damage/repair, but is modulated through specific signal transduction pathways. This signal transduction mechanism is now the focus of substantial research: looking for the UV-B photoreceptor(s), identifying signal transduction components and investigating the interaction with the plant genome. Understanding these complex networks will provide valuable insights to a variety of UV-B responses including plant defence mechanisms, species variation and interaction with other environmental stress factors. With the application of modern tools of molecular biology such as DNA-chip technology, new insights are being gained. In this brief presentation, I will review our understanding of UV-B responses at the molecular level and identify areas for future exploration.

11.13.3. Plant morphological responses to UV-B: mechanisms and ecological consequences

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Exposure to ultraviolet-B radiation (UV-B; 280-320 nm) at ambient or enhanced levels can result in a wide variety of morphological alterations in higher plants, but the mechanisms and ecological significance of these changes remain poorly understood. Results from laboratory studies indicate that certain morphological responses to UV-B (stem elongation) involve multiple sensory systems. In dim-red light grown and white-light grown seedlings of cucumber, short-term exposure to full-spectrum UV-B (280-320 nm) and long wavelength UV-B (300-320 nm) both induced inhibition in hypocotyl elongation but treatments differed in the timing and duration of the responses, dose responses, reciprocity adherence, and association with DNA damage/repair. Results from multi-species canopy simulations indicate that subtle changes in shoot morphology can lead to large shifts in interspecific light capture, and more so for mixtures of broad-leaved than narrow-leaved species. Alterations in the ratios of UV-B:UV-A:PAR occur within canopies and these spectral changes have the potential to modify the effects of UV-B on shoot morphology and competitive interactions.

11.13.4. Sensitivity of pasture plants to UV-B radiation and interaction with drought

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During summer, high levels of ultraviolet-B (UV-B) radiation frequently coincide with periods of drought in New Zealand. White clover (*Trifolium repens* L.) is the key legume component of pasture ecosystems in New Zealand, where it usually grows in association with perennial ryegrass (*Lolium perenne* L.). We sought to evaluate responses to UV-B radiation and drought in white clover plants grown separately or together with ryegrass in a sward. Our results revealed significant interactions between the two stress forms, suggesting ameliorating effects of the UV-B x drought combination for white clover growth. Results in white clover leaves showed marked increases of flavonoid levels, particularly quercetin glycosides, in response to UV-B and drought. Furthermore, our findings link accumulation of quercetin glycosides to UV-B protection. We are currently using comprehensive metabolite analysis (metabolomics) to identify other key plant compounds that can be used for the development of stress-resistant forage cultivars.

11.13.5. Ambient UV radiation levels negatively affect the endemic Antarctic moss, *Grimmia antarctici*.

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Over recent decades, Antarctic plants have been exposed to the largest relative increase in UV-B exposure as a result of ozone depletion. Open-sided plexiglass screens were used to reduce UV levels by 77% over turves of the endemic Antarctic bryophyte, *Grimmia antarctici*, and the effect on pigment concentrations, surface reflectance and physiological and morphological parameters was investigated over 14 months. *G. antarctici* was affected negatively by ambient levels of UV radiation. Chlorophyll content was significantly lower in plants grown under near ambient UV, whilst the relative proportions of photoprotective carotenoids, especially β -carotene and zeaxanthin, increased. No evidence for accumulation of UV-B absorbing pigments in response to UV radiation was observed. Although photosynthetic rates were not affected, there was evidence of UV effects on morphology. Given that other Antarctic bryophytes possess UV-B absorbing pigments that should offer better protection under ambient UV-B radiation, these findings suggest that *G. antarctici* may be disadvantaged under a climate with continuing high levels of springtime UV-B radiation.

11.13.6. Effects of supplemental UV-B radiation on photochemical activity, photosynthetic pigments and UV-B-absorbing compounds in two oak species

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The aim of this work was to compare the photosynthetic responses of two tree species (*Quercus petraea*, *Quercus robur*) and to determine the leaf level protection under enhanced UV-radiation in outdoor experiments. Three-year seedlings of both species were exposed to enhanced UV-B radiation (40 % of the ambient level) from bud-break until leaf senescence. Under enhanced UV-B, increases of specific leaf mass (SLM) and reduction of leaf chlorophyll content were observed but the total carotenoid content remained high. Both oak species responded with an increase of the VAZ pool to enhanced UVB. High values of potential photochemical efficiency (Fv/Fm) were maintained in both species during the vegetation season. On clear days, loss of Fv/Fm was observed from morning till midday which was larger under enhanced UV-B for both species, suggesting that UV-B exposure enhanced the sensitivity of seedlings to photoinhibition. Enhanced UV-B increased the amount of UV-B absorbing compounds in leaves of *Q. robur*, but it only slightly affected these levels in leaves of *Q. petraea*.

11.13.7. Impacts of ambient solar UV (280-400 nm) radiation on three tropical legumes

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Tropical regions currently receive the highest level of global solar ultraviolet (UV) radiation, especially UV-B (280-320 nm). The high level of solar UV-B radiation reaching the earth's surface is an important environmental concern. The average daily dose of UV-B radiation in Madurai, South India (10° N) is 10 KJ m⁻². This is approximately 50% more than the average daily UV-B radiation in many European countries. A field study was conducted using selective filters. Either the UV-B (< 320 nm) or UV-B & A (<400 nm) portions of the solar spectrum was removed and the effects were followed in *Cyamopsis tetragonoloba*, *Vigna mungo* and *Vigna radiata*. When compared to ambient radiation, exclusion of solar UV-B increased seedling height, leaf area, fresh weight and dry weight, and the crop yield by 50% in *Cyamopsis tetragonoloba*. The extent of this increase was slightly less under UV-B & A exclusion. In *Vigna mungo* a significant reduction was seen in solar UV excluded plants, while *Vigna radiata* was found to be unaffected.

11.14.1. Biosynthesis of indole and benzoxazinones in *Zea mays*

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Plant secondary metabolites constitute a large field of chemical biodiversity that is important for the survival strategies of plants. A secondary metabolic pathway can be defined by the branchpoint from primary metabolism and the downstream reactions that lead to end product formation. Evolution has generated specific enzymes that catalyse these reactions. The formation of indole branches off from tryptophan biosynthesis and is catalysed by enzymes resembling tryptophan synthase alpha subunits. In maize, indole can either function as volatile signal for communication with insects or be converted into benzoxazinoids by at least eight specific enzymes. Benzoxazinoids function as general plant defence chemicals. Gene duplication was essential for the evolution of the genes encoding these pathways. The examples show that gene duplication does not necessarily result in redundancy, rather it is a prerequisite for the generation of biodiversity.

11.14.2. Gene structures and functional diversification of enzymes of secondary metabolism in *Lotus japonicus*

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Leguminous plants have a diverse array of secondary metabolites with eco-physiological functions. To gain insights into their molecular evolution, we analyzed the gene structures in a model legume, *Lotus japonicus*, and catalytic activities of enzymes of legume-specific and general (iso)flavonoid pathway and oxidosqualene cyclases (OSCs) constructing triterpenoid skeletons. A total of four and five genes of chalcone polyketide reductase in the 5-deoxy(iso)flavonoid biosynthesis and dihydroflavonol reductase (DFR) involved in anthocyanin/condensed tannin pathway, respectively, constituted tandem clusters. Functional diversity of DFR isozymes was correlated to an amino acid variation in a region reported to control the substrate preferences. Three chalcone isomerases (CHIs) producing 5-deoxyflavonoids and one general CHI were also present as a tandem gene cluster. Eight OSCs compose two sets of gene clusters, and exon length polymorphism was found to be responsible for the product variations. Local gene duplication and specific mutation of the genes were thus indicated to be critical in the diversification of secondary metabolism.

11.14.3. Pinoresinol-lariciresinol reductases with different stereoselectivity

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Pinoresinol-lariciresinol reductases (PLRs) which are involved in lignan biosynthesis form together with phenylcoumaran benzylic ether reductases (PCBERs) and isoflavone reductases (IFRs) an evolutionary strongly related group of reductases [Gang et al. (1999) JBC 274, 7516-7527]. Whereas PLRs and IFRs work enantiospecifically PCBERs do not. We could isolate DNA sequences encoding PLRs from lignan accumulating suspension cultures from *Linum album* and *L. usitatissimum*. The proteins which were heterologously expressed in *E. coli* show opposite enantiospecificity with respect to the substrate pinoresinol and the product secoisolariciresinol. Mutants of the PLR from *L. album* which are generated by site-directed mutagenesis and expressed in *E. coli* are under investigation to figure out the molecular reasons for the opposite enantiospecificity. Furthermore, we could isolate two sequences encoding PLRs beside six further sequences probably encoding PCBERs from *Arabidopsis thaliana*. Data about the activity of the heterologously expressed proteins will be presented.

11.14.4. Divergent evolution in the flavonoid dioxygenase family

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Plants classified to the Apiaceae family are known to accumulate various flavonoids belonging to distinct subclasses. Four 2-oxoglutarate dependent dioxygenases, flavone synthase I, flavanone 3 β -hydroxylase, flavonol synthase and anthocyanidin synthase, are involved in the biosynthesis of these secondary metabolites. We have cloned genes encoding putative dioxygenases from several plants of Apiaceae family. The unique occurrence of flavone synthase I in this family and the high sequence similarity towards flavanone 3 β -hydroxylase is of special interest in evolutionary context. Functional identification of cDNAs from Ammi majus, Anethum graveolens, Apium graveolens, Pimpinella anisum, Conium maculatum and Daucus carota coding for either flavone synthase I or flavanone 3 β -hydroxylase, followed by molecular and phylogenetic analysis of the obtained sequence data, were performed to extend our knowledge of Apiaceae 2-oxoglutarate-dependent dioxygenases. After analysis of the data we postulate that FNS I results from a gene duplication of FHT and functional diversification during evolution.

11.14.5. Molecular cloning and expression of progesterone 5 - reductase from *Digitalis lanata* Erh.

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LS Pharmazeutische Biologie, Erlangen, Germany.

Digitalis lanata Ehrh. is an important medicinal plant. Cardiac glycosides isolated thereof are used in the treatment of cardiac insufficiency in man. *Digitalis* glycosides are mainly synthesized and accumulated in the rosette leaves. Although their biosynthesis has been studied extensively, molecular biology approaches have only been tried for few cardenolide biosynthesis specific enzymes and their corresponding genes. We have isolated a cDNA clone from *Digitalis lanata* leaves that encodes a progesterone 5 - reductase (5 -POR). 5 -POR catalyses the conversion of progesterone to 5 -pregnane-3,20-dione, a putative precursor of all 5 -configured cardenolides. Over-expression of the protein was achieved in *E. coli* using the pQE expression vector system. The purified recombinant protein showed 5 -POR activity. Meanwhile, 5 -POR cDNA clones have been isolated from 9 *Digitalis* species, and it was demonstrated that this gene is highly conserved within the genus. 5 -POR cDNAs were also isolated from 4 *Isoplexis* species, and our data support the opinion that *Isoplexis* and *Digitalis* should be combined into one genus. Evolutionary aspects will be discussed.

11.14.6. A possible evolutionary link between camalexin from *Arabidopsis* and a topoisomerase-inhibitor from *Streptomyces*

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The evolution of secondary metabolites of similar chemical structure in distantly related organisms is poorly understood. 3-Thiazol-2'-yl-indole (camalexin) is the major phytoalexin of *Arabidopsis*. Camalexin biosynthesis involves the cytochrome P450 genes CYP79B2 and CYP71B15, which are temporally and spatially induced after pathogen challenge. A biologically active derivative of camalexin (BE 10988) is synthesized by a *Streptomyces* strain. We investigate whether the biosynthesis of this derivative shares common intermediates of camalexin biosynthesis and also involves cytochrome P450s, which are mechanistically comparable to the corresponding plant enzymes. In *Streptomyces*, cystein conjugation is most likely catalyzed by a non-ribosomal peptide synthase, which is a target to isolate BE 10988 biosynthetic genes. As cystein is universally present, conjugation of secondary metabolites with cystein could involve enzymatic domains conserved between plants and bacteria.

11.14.7. Microevolution of toxin synthesis in cyanobacteria

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Cyanobacteria do occur frequently in surface water and contain a high diversity of bioactive compounds. Typically isolates of the same species differ in composition of bioactive peptides but cannot be discriminated in the microscope nor by traditional taxonomic markers (i.e. 16S rDNA). Those peptides are formed by modular multienzymes which belong to the largest enzymes inside the cell. The ecological mechanisms affecting existing pathways of

secondary metabolite synthesis are not understood. We have identified DNA rearrangements and mutations within gene regions (mcy) involved in the synthesis of the toxic peptide microcystin (MC) which are either linked to specific structural variants differing in bioactivity or complete inactivation of the total mcy gene cluster (55 kb). Inactive mcy genotypes have been found in relatively high numbers in lakes. Insertions found within the inactive mcy gene cluster contained conserved domains assigned to transposable elements. New structural variants specific to populations have been found. It is speculated that transposable elements can explain the abundance of mcy mutants and DNA rearrangements in our lakes.

11.15.1. The effects of tree species diversity on nutrient cycling and ecosystem productivity

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Tree species have different growth efficiencies and capacities to acquire and recycle nutrients resulting in specific productivities and effects on the nutrient cycle. Mixing species may simply create a patchwork pattern of these specific effects, or it can result in synergistic or antagonistic effects between species. This review focuses on the latter: mechanisms responsible for changes in forest productivity that result from the interactions of tree species in mixed stands. In particular, we will focus on competition, facilitation and competitive reduction, and the species attributes and processes that produce these interactions. The effects of species mixtures on nutrient acquisition, cycling and losses will be reviewed, and the relative importance of diversity on the one side and specific species attributes on the other side examined. In addition, experimental approaches and methods that can be employed to investigate the effects of increasing tree diversity will be discussed.

11.15.2. Spatial heterogeneity of abiotic parameters in Kakamega Forest: a process that preserves tree diversity

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Canopy structure defines abiotic parameters such as light, water, and nutrient distribution in the understorey. These parameters characterize the niches for the seedling survival and establishment. If a highly diverse forest specifies more different niches than a less diverse forest, more species should be able to establish in the highly diverse forest and thus the diversity preserves itself. Amount and variability of throughfall and its chemical properties were measured since September 2001 on a grid based design in 9 plots with 9 rain collectors each. Tree species composition was evaluated. Hemispherical images were taken to describe the canopy structure with parameters such as canopy openness, LAI, and radiation. Spatial heterogeneity of rainfall exists on and between all plots with different values of heterogeneity in the plots triplets and different amounts of throughfall precipitation. The variance of the precipitation of the plots is compared with the total variance of precipitation and with vegetation structure characteristics such as light values and tree diversity.

11.15.3. Woody invasive species increase nutrient cycling in the granitic Seychelles

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Geobotanical Institute, Zürich, Switzerland.

Invasive plant species tend to have larger specific leaf area (SLA) and higher nutrient concentrations compared to native species. These properties may lead to faster litter decomposition and increased soil nutrient availability. To test for such impacts, we compared leaf litter properties and decomposition rates of 6 native and 6 invasive woody species in forests of the granitic Seychelles. Litter bags were incubated in the field and in the greenhouse. Nutrient availability in soil was determined under stands of two invasive (one N-fixing) and one native species. Tree seedlings were grown in pots with these soils to assess soil fertility and nutrient limitation. As hypothesised, litter of invasive species had higher SLA and nutrient content than native litter and decomposed faster. We found indication of elevated viz. reduced (N-fixing

species) available P in soils under invasive species. The phytometer experiment suggested that seedling growth in these soils is P-limited, and that P-limitation is alleviated under one invasive species. This may cause a positive feedback since invasive woody species in the Seychelles thrive best on nutrient-rich soils.

11.15.4. Different pollination efficiency and outcrossing by birds and bees in *Metrosideros excelsa* (Myrtaceae)

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Loss or modification of native pollinator guilds can have profound effects on plant reproductive output. Exclusion experiments were used to assess the effect of birds and bees on seed production and outcrossing in *Metrosideros excelsa*, a New Zealand tree species that has lost several indigenous bird pollinators on the mainland. Native birds are more important pollinators of *M. excelsa* than native bees. On Little Barrier Island, an island off the mainland with an original contingent of indigenous birds and bees, the number of fertile seeds per capsule was 48% higher after open pollination than in treatments with bee visitation only and 34% higher than treatments allowing autonomous self-pollination only. Estimated outcrossing rates were higher ($t_m = 0.71$) for open pollination in the upper canopy than for the treatment with bee access only ($t_m = 0.40$). In a modified mainland population with predominantly introduced birds and bees, seed production for all treatments was higher than on Little Barrier Island, indicating that introduced flower visitors and autonomous self-pollination are now important for seed production in *M. excelsa* on the New Zealand mainland.

11.15.5. Effects of tree stand diversity on insect herbivory: results from several long-term experiments in boreal and temperate forests

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We examined the effects of tree stand diversity on insect herbivory in several long-term experiments in Finland, Sweden and England where tree species diversity of stands has been manipulated. In the Finnish experiments, insect herbivory on silver birch was higher in birch monocultures than in birch mixtures with Scots pine, but only at the beginning of the season and only in mixtures which contained 75% of pine. No effects of stand diversity were found on insect herbivory on silver birch growing in monocultures or in mixtures with Scots pine and Norway spruce in the Swedish experiment. In the second rotation of the Gisburn forest diversity experiment (NW England), insect herbivory on sessile oak and black alder was lowest in monocultures and particularly high in oak-alder mixtures. We conclude that the effects of forest stand species diversity on insect herbivores vary from positive to negative depending on the type of herbivores present, the identity of tree species constituting a mixture, time of the season and geographic locality. In other words, forest diversification does not provide a universal remedy for insect pest problems.

11.15.6. A global network of biodiversity experiments in forests

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During the last five years, several attempts have been made to establish large-scale and long-term experiments designed to examine the effects of enhancing tree diversity in plantation forests on ecosystem functioning and to identify underlying mechanisms. These projects are based in Germany, Finland, Panama and Borneo. Each of these experiments comprises an experimental diversity gradient of large plots planted with different numbers and types of tree species. The presentation will give overviews of the experimental designs and of the first results, focusing on the BIOTREE (BIODiversity and ecosystem processes in experimental TREE stands) experiment in Germany. In that project, 81 plots covering more than 80ha were afforested with trees of varying species richness and functional diversity on three different sites. In total, 250,000 saplings have been planted. Several ecosystem processes will be monitored regularly, with an emphasis on tree growth, carbon sequestration, and nutrient cycling.

11.16.1. Application of Bioindication and Biomonitoring as Innovative Biotechniques for Pollution Control Measurements

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Bioindicators reflect organisms (or parts of organisms or communities of organisms) that contain information on quality of the environment. When data and information obtained by bioindication are moved up to the level of knowledge the subjectivity of interpretation increases with the complexity and dynamics of a system ("staircase of knowing"). From there a comparison of instrumental measurements with the use of bioindicators/biomonitoring with respect to harmonisation and quality control will be drawn. Precision, accuracy, calibration and harmonisation in between national standards and international routines seem to be the leading goals in quality studies of international working groups dealing with biomonitoring throughout the world. The newly developed Multi-Markered-Bioindicator-Concept (MMBC) will fill the gap in between human health aspects or environmental protection purposes. Markert, BA., Breure, AM. and Zechmeister, HG (eds.) (2003) *Bioindicators & Biomonitoring*. Principles, Concepts and Applications. Elsevier, Amsterdam, pp. 998.

11.16.2. Monitoring the effects of air pollution and nitrogen deposition by the diversity of epiphytic lichens and correlation with the incidence of respiratory cancer mortality in central Italy

S. Loppi;
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In field monitoring studies, it is very difficult to separate the effects of many intercorrelated variables, and the interpretation of lichen biomonitoring surveys in terms of air pollution is open to some controversy. It is well known that lichens are sensitive to air pollution, however, other ecological variables such as climate and substratum also induce variations in the frequencies of lichen species. As a consequence, it is not always easy to discriminate between the effects of pollution and those of other environmental changes. Now that SO₂ is no longer the dominant air pollutant, the total lichen diversity seems to have lost its predictive value for air pollution, and when using the diversity of epiphytic lichens for monitoring environmental pollution, the interpretation of the results could consist of several maps, each of which based on different groups of species, and each of which interpreted in a different way. Several examples from central Italy will be given to show all these situations, including correlations of lichen diversity counts using reduced datasets of selected species with the incidence of respiratory cancer mortality.

11.16.3. Stress effects of NH₄ deposition in *Thymus pulegioides* L. using a biomarker pattern of amino acids as indicator

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A 24 days experiment on *Thymus pulegioides* L. species was performed, simulating NH₄⁺ deposition and soil eutrophication. The aqueous solution of urea was used as NH₄⁺ source, in serial concentrations of 6,5 g/L, 13 g/L, and 26 g/L, added at once in the soil. Thin Layer Chromatography and High Performant Planar Chromatography methods were used in order to assess the sensitivity and stability of phytochemical changes (biomarker pattern) following the exposure. The content of glutamine, serine, alanine, valine and isoleucine showed positive correlation with the urea concentrations applied. The correlation time span from exposure/ amino acid content showed different behaviors depending on each amino acid. The biomarker pattern was correlated to the morphological and ecophysiological changes exhibited by plants. A stimulation of plant growth was observed at the highest exposure concentration, but the biomarker pattern appeared very strong even for the lowest exposure concentration, when no visual signs were detectable. The possible use of biomarker pattern method as an "early warning system" related to the NH₄⁺, NH₃ or other forms of N deposition is discussed.

11.16.4. Botany and public health - worth of plant species richness to human health

A. K. F. Malsch, C. Hornberg;
University of Bielefeld, Bielefeld, Germany.

Environmental (public) health matters ranges from environmental medicine (comprises human toxicology and environmental epidemiology) to holistic approaches in human ecology. All research areas increasingly support preventive strategies and health promotion, since globalisation uncovers the dependency of human health from ecosystem health and in particular from plant species richness. Plants provide many important ecosystem-services: 1) utility services (indicators, food, clothes, pharmaceuticals, oxygen e.g.), 2) protection services (flood and erosion control, buffer function e.g.) and 3) recreation services (active or passive relaxation in "green" nature). Sustainable ecosystem management is pre-condition for human health protection and promotion. This holds true especially in urban areas where natural recreation areas are most important from ecological and public health perspective. There are manifold interdisciplinary research interfaces between ecology and public health waiting to be tackled.

11.16.5. Marine macroalgae as indicators of metal contamination in seawater: active and passive biomonitoring, metal uptake and chlorophyll fluorescence approaches

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Traditionally representatives of the Phaeophyta, Chlorophyta and Rhodophyta have been used as biomonitors for marine metal contamination, even though metal concentration factors differed greatly between different species. In addition, it is now accepted that metal accumulation is influenced by species-specific and seasonal growth patterns, biochemical composition (e.g. phenol and polysaccharide contents and composition), as well as ambient environmental conditions affecting metal bioavailability. To evaluate the potential of marine macroalgae as both sensitive and reliable biosensors for metal contamination in seawater, different species were applied as passive biomonitors (analysis of metals in algae from different locations, different thallus parts and different species within sites) and active biomonitors using transplantation techniques. Metal uptake properties of different macroalgae were compared under different temperature and salinity regimes. In vivo chlorophyll fluorescence (PAM-2000, Walz, Germany) was used to quantify metal toxicity of different species during and after exposure to metals at different concentrations.

11.16.6. Phytotoxicity tests to be used in ecological risk assessment of heavy metal polluted soils

P. Cesaro, A. Volante, N. Massa, E. Bona, G. Lingua, G. Berta;
Università del Piemonte Orientale "A. Avogadro", Alessandria, Italy.

Plants respond to metal toxicity by eliciting a number of bio-marker endpoints that can be detected at various levels of organization, ranging from gross morphology to organ, cellular and molecular levels. We analysed the effects of the soil of two industrial sites situated in the provinces of Alessandria and Savona (Italy) on *Pisum sativum* plants. In addition to the conventional germination tests and root growth analyses, we calculated the percentages of dividing mitotic cells (mitotic index), of the mitotic phases in the root apices, and of the mitotic anomalies. DNA loss and damage were evaluated by flow cytometry and comet assay, respectively. Lower mitotic indices, an increase of the percentages of prophase and mitotic anomalies (as sticky or broken chromosomes, and bridges in anaphases) were found. DNA loss was also detected in treated plants and comet tests were strongly positive, suggesting DNA damages. All together, our results showed a high level of toxic substances in the soil and suggest the use of the described biotests (highly sensitive) in evaluating soil pollution.

11.16.7. Metadata and classification and regression trees on accumulation of metals in mosses

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The UNECE Metals in Mosses Surveys provide exposure data for ecotoxicological risk assessments by measuring the accumulation of metals. Measurement data and metadata from the German Surveys 1990, 1995 and 2000 are analysed with regard to the following hypotheses: 1. The metal accumulation in mosses is correlated with the distance of the sampling sites to emission sources. 2. The metal loads are correlated with the distance between the sampling points and trees because of canopy drip effects. 3. Topographical features like inclination, exposition and

altitude cause spatial variability of the accumulation. 4. Moss species accumulate metals specifically. 5. The accumulation varies with ecoregions. The metadata describe the measurement sites with respect to some of those surrounding environmental conditions that could be relevant for the spatial variances of the metal accumulation in mosses. The statistical design to investigate these hypotheses includes GIS-techniques, contingency tables and correlation analysis, geostatistics as well as Classification and Regression Trees (CART). CART is used for a multivariate typology of the metal accumulation.

11.17.1. Invasive alien plant species and biodiversity changes: a local perspective.

B. E. Tokarska-Guzik, **B. M. Wegrzynek**, A. Urbisz, T. Nowak, A. Urbisz;
University of Silesia, Katowice, Poland.

The aim of the study is to describe the impact of invasive plant species on the native flora and vegetation with respect to particular phytogeographical region of Poland. The researched area was the Silesian Upland (southern Poland) which is considered significantly species rich and diverse. At the same time it is still perceived both in Poland and Europe as an ecological disaster area with a strongly distorted and modified natural environment. The current flora of the Silesian Upland has been investigated by using the method of mapping of all the species on a grid of equal basic fields: squares with a side of 2 kilometres. A separate list of the flora of each study square was prepared on the basis of floristic notes and phytosociological relevés. For alien plant species population size and type of habitat as well as plant community invaded were recorded. The results of the studies have shown the composition of the spontaneous flora of the region, participation of alien plant species and reflect the spatial and habitat diversity in the area. There was also an attempt to propose the first regional list of invasive alien species, which threatened native flora and vegetation.

11.17.2. Alien invasive plants in natural and semi-natural habitats from Romania

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Based on information from literature, herbaria collections, as well as on field researches, and taking in account the recommended terminology in ecology of plants invasions, we identified 435 alien plants in Romanian Flora. We consider that 38 taxa are invasive in different types of ecosystems. Among these, 24 taxa affect natural and semi-natural habitats. In this paper we discuss the impact of these plants on this kind of habitats from different regions of our country, as well as from some natural reserve: National Park Muntii Macinului, Natural Park Bucegi (important center for endemic species), Natural Park Portile de Fier. So, while some do not produce important modifications, others change the character or the nature of some ecosystems (*Azolla filiculoides*, *Paspalum paspalodes* etc.). Some invasive alien plants are integrated in natural landscape so that, based on them, plants associations that are new for science could be described (*Iva xanthifolia* etc.).

11.17.3. Weeds and invasives in Mexico - What is new?

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Mexico and adjacent Central America are a center of agricultural origin. It is the home of numerous domesticated plants, and of many widespread weeds, particularly of the tropics. In the past 10 years, the study of the weed flora has advanced considerably, both from regional work, as from the analysis of data in a database of the country's flora. Mexico has almost 3000 registered weed species, out of 23,000 higher plants for the whole country. Only 2.7% (617) are exotic, despite a high level of diaspora pressure for 500 years. We show that a) a positive simple correlation between species richness of native and exotic weeds turns negative if factors that influence diversity in general are excluded, supporting Elton's biotic resistance hypothesis; b) exotic species have smaller average distributions than native weeds; so, most exotic species are not intrinsically superior nor have a enemy escape advantage, or are still in the lag phase; c) families differ considerably in their

proportion of native weeds vs. exotics, so differential relative advantages must exist (taxonomic hypothesis). - The presentation also introduces an internet Flora of the weeds of Mexico.

11.17.4. Biotic homogenization of the California flora

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Biotic homogenization is driven by native species losses and invasive species gains. Patterns of homogenization were investigated for the flora of California. Data from a variety of available databases were aggregated at the county level to examine patterns in county population density and growth as well as floristic change. Examining patterns of rare plant occurrences among these counties revealed that high and medium density counties contained, on average, as many or more rare and endemic species than low density counties. Over 48% of the 962 highly threatened taxa in California are endemic to the high and moderate density counties. Thus, urban and urbanizing counties play a large role in maintaining that part of California's flora that is both unique and threatened with extinction. Examining species losses and noxious weed additions across high density counties, reveals a consistent pattern of low similarity among species that have been extirpated from high density counties and a high similarity among noxious weeds that they all now share. The consequence is that California county floras in urban areas are becoming more homogeneous.

11.17.5. Alien invasion and habitat disturbance: effects on pollination and reproduction in a native boreal forest herb

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Norwegian University of Life Sciences, Ås, Norway.

The impacts of alien plant species on pollinator visitation and reproductive success of native species has been little studied, and no studies have examined such impacts in habitats of contrasting disturbance history. We studied how experimental introductions of an alien species (*Phacelia tanacetifolia*) affect pollinator visitation and female reproductive success of a native (*Melampyrum pratense*) in recently logged and in old-growth boreal forest habitats. *Phacelia* significantly increased the number of bumblebees entering plots in both habitat types. However, the alien species had a strong negative impact on visitation rate of the native species in both habitat types. Despite this negative impact on pollinator visitation, the alien had no effect on female reproductive success of the native species in any habitat. Our results show that reproduction may be more robust to alien invasion than pollinator visitation, and that impacts of alien invasion may not differ between habitats of contrasting disturbance history.

11.17.6. Ecological status of the invasive exotic weeds in Shivalik Ranges of Northwestern Himalayas, India

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Panjab University, Chandigarh, Chandigarh, India.

Northwestern Himalayas of India have a rich floral diversity. Unfortunately, during the past few years there has been an increase in number of invasive exotic species that have either been introduced deliberately or have entered accidentally. A numbers of factors are responsible for their fast spread such as increasing inter/intra continental links, import/export activities, and climatic changes. It has resulted in a dramatic shift and decrease in the diversity of native flora. Lack of awareness, insufficient information on the species and their respective dimensions of spread and behaviour couple with the lack of coordination and collaboration has further compounded the problem. It is proposed to review the current status of exotic plant species, their range of distribution and impact on the diversity and richness of the native ecosystem health. In addition, some strategies for control of their spread and regular monitoring of the vegetation structure and succession mechanism of the region shall also be discussed.

12.1.1. Elucidating the small RNA component of the transcriptome with massively parallel signature sequencing

P. J. Green¹, C. Lu¹, S. Tej¹, S. Luo², C. D. Haudenschild³, B. C. Meyers¹;

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Small RNAs play important regulatory roles in most eukaryotes but only a small proportion of these molecules have been identified. We have developed a novel method for the identification and measurement of small RNAs that provides a substantial advance over existing methods for the analysis of these RNA molecules. This method is based on massively parallel signature sequencing ("MPSS") and can sequence hundreds of thousands of molecules in parallel. From two libraries representing *Arabidopsis* flower and seedlings, we identified more than 75,000 different small RNA sequences. We are combining experimental and bioinformatics approaches to gain insight about the regulation, genomic distribution and roles of these molecules. This powerful genome-wide approach extends miRNA prediction capabilities and is applicable to diverse organisms. Funded by the National Science Foundation.

12.1.2. Antisense transcript and RNA processing alterations suppress instability of polyadenylated mRNA in *Chlamydomonas* chloroplasts

Y. Nishimura, E. A. Kikis, S. L. Zimmer, Y. Komine, D. B. Stern; Boyce Thompson Institute for Plant Research, Ithaca, NY, United States.

In chloroplasts, the control of mRNA stability is of critical importance for proper regulation of gene expression. The *Chlamydomonas reinhardtii* strain delta 26pAtE is engineered such that the *atpB* mRNA terminates with an mRNA destabilizing poly(A) tract, resulting in this strain being unable to conduct photosynthesis. A collection of photosynthetic revertants (suppressor of polyadenylation (*spa*)) was obtained. Among them, *spa19/23* maintained unusual two heteroplasmic chloroplast genomes (PS+ and PS-). Based on RT-PCR, S1 nuclease protection, evidence was obtained that the PS+ genome stabilizes polyadenylated *atpB* mRNA by generating an *atpB* antisense transcript. Furthermore, when a 121-nucleotide segment of *atpB* antisense RNA was expressed in delta 26, in which *atpB* mRNA is unstable because of the lack of a 3' stem-loop structure, an elevated accumulation of *atpB* mRNA resulted. Taken together, our results suggest that antisense RNA in chloroplasts can protect otherwise unstable transcripts from 3'→5' exonuclease activity.

12.1.3. Pre-mRNA splicing in plants: SR proteins and interacting modifying proteins

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Pre-mRNA processing is an important step in gene expression and its regulation leads to the expansion of the gene product repertoire. As SR proteins are the important group of proteins in defining intron borders we have isolated and characterized about a dozen of these important proteins which are organized in several protein families. Characterising their expression pattern by several techniques, differential expression within a family indicate evolution of their different regulation. Ectopic overexpression of particular members of each family show their specific involvement in auxin and sugar metabolism and light /dark responses. By yeast two-hybrid screening several new plant splicing factors could be isolated and their interactions verified by pull-down and immunoprecipitation assay. Interestingly, proteins which fuel the dynamics of spliceosomal assembly, like SR specific kinases and cyclophilins (peptidyl prolyl isomerases) were found by screening and further characterized. Specifically, CypSR64 was found to act early in spliceosome assembly whereas Cyp55 could possibly function at the interphase of transcription and pre-mRNA splicing.

12.1.4. RNA processing in *Arabidopsis* flowering time control

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Arabidopsis flowering is repressed by the action of the MADS box transcription factor, FLOWERING LOCUS C (FLC). The accumulation of FLC mRNA is controlled by the genetically-defined autonomous pathway. This pathway includes the novel, plant-specific, RNA binding proteins, FCA, FPA and FLK. These novel RNA binding proteins deliver precision to the regulation of FLC expression, but how do they function? FCA interacts with the RNA 3' end processing factor, FY. This interaction is required for FCA to autoregulate its own expression and to negatively regulate accumulation of FLC mRNA. FCA autoregulates its expression by promoting premature cleavage and polyadenylation within intron 3

of its own pre-mRNA. We are currently characterizing the mechanism by which FPA controls flowering. FPA comprises three RRM-type RNA binding domains and a novel, plant-specific domain found in other plant RNA binding proteins. Genetic studies indicate that FPA controls flowering time independently of FCA.

12.2.1. *Physcomitrella patens* as a novel tool in plant functional genomics

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Mosses are characterized by a simple body plan, and haploidy of the major tissues. *Physcomitrella patens* displays homologous recombination in its genome, enabling efficient reverse genetics. An EST database was established covering over 95% of the 30,000 protein-encoding genes. The draft genome will be published later in 2005. Several thousand genes are not known from seed plants, hundreds of them appear as retained genes. The set of transcription factors was identified and compared to the complete set of TFs in *Arabidopsis*. Efficient protocols for proteomics approaches were established including the genome-wide analysis of reversible protein phosphorylation. Insights from a collection of tagged mutants (75,000) as well as from single knockout-plants will be presented, including in-vivo imaging via CLSM and FRET.

Supported by DFG, BMBF, Fonds der Chemischen Industrie, and BASF Plant Science. Genome sequencing is performed by US DoE at JGI.

12.2.2. Parameters affecting the frequency of homologous recombination in *Physcomitrella patens*.

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Constructs in which a selection cassette is inserted into a length of genomic DNA have been used to target a number of loci, and the resulting transgenics have been analysed by PCR. The frequency of the occurrence of homologous recombination (HR) is strongly related to the log. of the length of the homologous sequence and is not affected by the relationship (5' or 3') to the selection cassette, nor do PCR-generated targeting sequences give different frequencies for the corresponding targeting sequences released from plasmids by restriction enzyme digestion. Gene targeting may result in allele replacement with junction sequences consistent with HR having occurred in both flanking sequences, but can also result in the insertion of the targeting sequence without replacement of a genomic sequence, by a process that involves not only HR but also non-homologous end joining (NHEJ). Sequence analysis indicates that NHEJ occurs between one or more molecules of the targeting sequence and does not involve the chromosomal sequence.

12.2.3. The genomics of desiccation tolerance in Bryophytes.

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The molecular, cellular and biochemical responses of plants to water-deficit stress are of major economic importance as we cope with a changing environment. We use the desiccation-tolerant moss *Tortula ruralis* as a model for studying post-transcriptional gene control, molecular & biochemical responses to abiotic stress, cellular repair mechanisms in plants and as a source of novel tolerance-associated genes. A major advance in our efforts have come with the development of a *Tortula* unigene set and a *Tortula* uni-cluster cDNA microarray. Analysis of expression profiles, over the desiccation rehydration cycle, and assessing both transcript abundance and recruitment into the protein synthetic pool has allowed us to develop a comprehensive look at the protection and repair aspects of desiccation tolerance in this bryophyte. Phylogenetic profiling, in this case bioinformatically, coupled with new phylogenetic assessments of desiccation tolerance in the land plants allows us to generate new testable hypotheses concerning the importance of certain genes and gene networks in the evolution of this important trait.

12.2.4. Stress- and ABA- responses in *Physcomitrella patens*: a transcriptomic approach

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Many mosses exhibit a strikingly high level of dehydration tolerance. The model moss *Physcomitrella* is able to withstand dehydrative loss of ca. 90% fresh weight and recover. Using a 22,000 feature oligonucleotide microarray designed from the *Physcomitrella* EST database, we have identified networks of genes co-regulated by dehydration, osmotic stress, salt stress and the plant hormone ABA. Analysis of a small number of such stress-regulated genes, encoding homologues of the higher-plant Late Embryogenesis Abundant (LEA) proteins, indicates a common pathway of transcriptional induction through evolutionarily conserved ABRE *cis*-acting promoter sequences. The potential of global transcriptional analysis through microarray studies, in combination with bioinformatic analysis of the emerging *Physcomitrella* genome sequence, is expected to enable the full scope of such gene co-regulatory networks to be revealed.

12.2.5. Phage-type RNA polymerases in *Physcomitrella*

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Mitochondrial and chloroplast genes of maize and other cereals are transcribed by two different RNA polymerases phylogenetically related to RNA polymerases of bacteriophages. Eudicots, like *Arabidopsis* and *Nicotiana*, possess an additional phage-type RNA polymerase that is dually targeted to both mitochondria and chloroplasts. Except certain non-green parasitic plants, transcription of chloroplast genes of all lower and higher plants additionally requires a bacterial-type RNA polymerase. Genes for subunits of the bacterial-type RNA polymerase reside in the chloroplast genome with the exception of a nuclear encoded *RpoA* gene in *Physcomitrella patens*. *Physcomitrella* shows another striking difference to angiosperms. The nuclear genome contains two genes for phage-type RNA polymerases, *PpRpoT1* and *PpRpoT2*. GFP-fusions of the transit peptides of the two polymerases revealed that *both* enzymes are dually targeted to mitochondria and chloroplasts. Attempts to knock out *RpoT1* in *Physcomitrella* failed, probably because it has an essential function. We obtained, however, knock-out mutants of *RpoT2* that exhibit a surprising phenotype.

12.2.6. Actin dynamics in *physcomitrella patens*: Role in polar tip growth

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We will describe how we employed two techniques in *P. patens*, i.e. homologous recombination and RNAi, to generate loss of function lines in order to determine the role of two different members of the Arp2/3 complex (a seven member protein complex required for nucleating and branching actin filaments) in polar tip growth. We first show that the transcript for ARPC1 and an ARPC4-YFP fusion protein are both localized at the tip of the elongating protonemal apical cell. Knockdown lines of ARPC1 and knockdown and knockout lines of ARPC4 both show abnormal polar tip growth phenotypes -lack of the extension growth. The ARPC1 abnormal phenotype is more severe; caulonemal cells are not formed resulting in the lack of buds and leafy gametophores. ARPC1 also appears to have a role in adjusting to osmotic stress and in establishing polar outgrowth. Latrunculin B, an actin-disrupting drug, mimics the severe ARPC1 phenotype in wild type protonema. We will discuss how these phenotypes will be used to further our understanding of how the alignment of polar tip growth is controlled. Funding: Helen Hay Whitney Foundation (M.B.); National Science Foundation (IBN 0112461 - R.Q).

12.2.7. *Physcomitrella*: at the intersection of bryology and pathology

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The ability to manipulate the genome of *Physcomitrella patens* makes it an attractive system for studying genes that regulate programmed cell death (PCD) and associated responses to microbial pathogens. Although the pathology of *Physcomitrella* (and bryophytes) is not well developed, it has the potential to become a model pathosystem for studies in molecular plant pathology. We describe several approaches for developing 'physcopathology', including the use of pathogens and elicitors to

induce defense responses and PCD. The perception of elicitors by *Physcomitrella* results in changes in secondary metabolites, the activation of defense-response genes and enzymes, and a stereotypical pattern of cellular breakdown. Manipulation of genes that regulate PCD affects both these responses and plant development. These results suggest that *Physcomitrella* can serve as a useful system for studying molecular aspects of PCD, plant defense responses and disease resistance.

12.3.1. Floral morphological character coding and the use of trees.

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Floral morphology has shed much light on phylogenetic relationships of taxa, and conversely, phylogenetic hypotheses have helped us interpret floral morphology. Because modern phylogenetic analysis requires us to explicitly define and code individual characters and character states, the results of phylogenetic analyses allow us to interpret more precisely the evolution of characters, to test the value of characters as evidence, and to refine definitions of characters. We will consider two character systems of the gynoecium that show the relationships between morphological interpretations of characters (as coded in the matrix) and the resulting outcome of the phylogenetic analysis. The concept of pseudomonomy has been assembled based on combinations of morphological and phylogenetic criteria. The phylogenetic interpretations must be removed when characters are coded for an analysis, but the coding and the phylogeny are inextricably linked when we discuss pseudomonomy. Morphological and phylogenetic interpretations of ovary position provide a similar example.

12.3.2. Floral structure and development in Malvaceae-Grewioideae

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Grewioideae, which include most genera of former Tiliaceae, represent a basal clade within Malvaceae. Their floral structure, especially the androecium, is unique within Malvaceae, where fertile stamens usually occur in antepetalous position, and staminodia in the antesepalous sector, which is interpreted as (ob-)diplostemony. In most Grewioideae the first staminal primordia arise in antesepalous position, followed by antepetalous primordia, which are slightly more peripheral. Additional stamens can arise centrifugally in the antesepalous sectors, less so in antepetalous position. *Sparrmannia* produces exclusively antesepalous stamens. Anatomical features are not always congruent with these ontogenetical observations: Whereas in *Corchorus* and *Trichospermum* only antepetalous traces could be found, there are antesepalous as well as antepetalous traces in *Grewia* and *Sparrmannia*. In *Sparrmannia* the antepetalous traces divide and supply stamens also in antesepalous position. Based on these observations the circumscription and use of the term 'androecial whorl' is discussed.

12.3.3. Floral Development and Floral Structure of Dipterocarpaceae

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The understanding of floral development within Malvales has become rather extended in the last years. However, one of the families not yet studied so far is the largely South East Asian Dipterocarpaceae. Organ primordia protrude from a strongly flattened floral apex. Sepal initiation is sequential. In Dipterocarpus the first two sepals become conspicuously enlarged later forming the two characteristic wings. The petals arise simultaneously and become contorted. In none of the investigated species a retardation of petal initiation was observed as shown in closely allied Cistaceae (Nandi 1998). Androecium initiation proceeds centrifugally. In Dipterocarpoideae, five episealous stamens are initiated simultaneously followed by two regular androecial whorls or, in taxa with >15 stamens (incl. Monotoideae, 1 species studied), male organs arise from a ring primordium. The gynoecium arises from three moulds that show a short synascidiate zone, which is also present in the anthetic flower.

12.3.4. Floral diversification in Spermacoce (Rubiaceae): evolutionary steps towards more efficient cross-pollination?

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Rubiaceae flowers feature impressive variation in size, shape and colour. The corolla bauplan, nonetheless, shows little variation. Most corollas are 4-5-merous, characterised by early sympetaly, and outgrowths are usually absent. In the Australian representatives of the genus *Spermacoce*, however, the corolla shows more variation. At the adaxial side of the corolla lobes of many species, outgrowths - called appendages in literature - are present, and in other species the number of corolla lobes is doubled. An ontogenetic study was undertaken in order to investigate the homology among the appendages observed. In addition, the species were studied in the field to illuminate the role of the corolla appendages to achieve more efficient cross-pollination. A phylogenetic hypothesis of the study group based on DNA sequence variation allows us to discuss the floral diversification in a phylogenetic context.

12.3.5. The most polymerous gynoeceum among asterids: floral development in *Tupidanthus calyptratus* Hook.f. & Thoms. (Araliaceae)

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The peculiar flowers of *Tupidanthus calyptratus* (= *Schefflera pueckleri* (K.Koch) Frodin) are characterized by very numerous floral parts (up to 172 stamens and 138 carpels), a condition of secondary origin unique not only within the order Apiales (where the pentamerous flowers are common and polymery is otherwise much less extreme) but within the asterids as a whole. The pedicels of *T. calyptratus* as well as of closely related *Schefflera* species bear two bracteoles. In *Tupidanthus*, the flower primordium is shaped like a butterfly or bow. The calyx is formed as a tiny ring, and sepal number is thus unclear. The corolla is initiated as a ring-meristem that is transformed into a massive entire tube-like structure with numerous distal lobes. The tube then curves inwards; its abaxial surfaces are postgenitally fused into a calyptra. All the primordia of the numerous stamens appear simultaneously as a single whorl, a mode of androecium initiation that is very rare within the Angiosperms. The carpels, initiated somewhat later than the stamens, likewise form a single whorl. A flat extensive central portion of the floral apex does not take part in the initiation of floral organs.

12.3.6. Flower morphology and development in former Melanthiaceae

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Molecular phylogenetic studies have supported division of the former Melanthiaceae s.l. into four relatively distantly related families: Melanthiaceae s.str. (Liliales), Nartheciaceae (Dioscoreales), Petrosaviaceae (Petrosaviales), and Tofieldiaceae (Alismatales). We have compared floral morphology and development in these families and their relatives. Common characters of all former Melanthiaceae are also present in many other monocots. Some features support the monophyly of each segregated family. However, each family displays significant variation of floral characters, including presence/absence of septal nectaries in Nartheciaceae, variation in ovary position in Petrosaviaceae, unilocular/trilocular ovary and variation in stamen number in Tofieldiaceae. Features supporting ordinal placements are often equivocal. For example, the characteristic calyculus of Tofieldiaceae is similar to the bract whorls in Alismatales (both Alismatales), but also to the leaf whorl that is inserted below the flower in *Trillium* and its allies (Liliales).

12.3.7. Bridging the basal eudicot and core eudicot boundary. How can we reconcile phylogeny with floral evolution?

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Most taxa of the basal eudicot grade, including Gunnerales, share simple dimerous flowers with little or no differentiation between bracts and tepals. The core eudicots have a majority of taxa with

pentamerous flowers and a clear distinction between sepals and petals. However, the understanding of the evolution of the perianth in the higher basal eudicots and the lower groups of the core eudicots is crucial for grasping the evolution and the differentiation of petals. It is demonstrated that on the one hand Gunneraceae represent an evolutionary dead-end, with a strong tendency for reduction of the flower linked with wind pollination. It is postulated that progenitors with spiral flowers similar to Berberidopsidaceae gave rise to pentamerous flowers with sepals and petals of a same tepaline origin. A progressive synorganisation of the flower linked with shifts in expression of genes determining organ identity led to a strong resemblance of petals to stamens in several higher orders of the core eudicots. Several reasons are postulated for the absence of any clear morphological links between the basal eudicot grade and the core eudicots.

12.4.1. horizontal gene transfer in the evolution of plant mitochondrial genomes

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I will first give a brief overview of the remarkable propensity of plant mitochondrial genomes to take up foreign DNA, from the chloroplast and nucleus via intracellular gene transfer and from other plant mitochondrial genomes via horizontal gene transfer. I will then focus on the latter topic, presenting recent work from my lab on the extent, frequency, functional consequences, and mechanisms of plant-to-plant horizontal transfer of mitochondrial genes. Special emphasis will be placed on the role of parasitism in horizontal transfer and on the basal angiosperm *Amborella trichopoda*, whose mitochondrial genome is exceptionally full of foreign genes, acquired from a wide range of angiosperm and moss donors. The implications - both negative and positive - of widespread horizontal gene transfer for the utility of plant mitochondrial genes as phylogenetic and chronological markers will be discussed.

12.4.2. Variation patterns in mitochondrial genes used in plant phylogenetics

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Mitochondrial genes, or more correctly, genes that usually occur in the mitochondrial genome, frequently exhibit variation patterns suggestive of marked changes in evolutionary rates. This phenomenon also is observed in the nuclear and plastid genomes, but comparisons across major clades of the angiosperms, and among major monocot groups in particular, using genes that have been widely sampled for phylogenetic purposes, suggest that it is more pronounced in the mitochondrial genome than in the plastid genome. Among the various patterns that are exhibited are those that suggest that the rate changes are persistent, and others that suggest episodic events of acceleration and deceleration. RNA editing, though directly inferred in only a few taxa, is correlated with variation patterns that can be used to infer the taxonomic distribution of this phenomenon. These patterns affect the results of phylogenetic analyses. Because they are not confined to the mitochondrial genome, and because this genome provides phylogenetic evidence that is not replicated in others, it would be inadvisable to exclude mitochondrial genes from phylogenetic analyses.

12.4.3. Evolution of RNA editing sites and its impact on phylogenetic signal in plant mitochondrial genes

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We present an analysis of sequence evolution and phylogenetic signal in three mitochondrial genes -cox1, matR, and atp1 - that we have sampled from diverse flowering plant species. Phylogenetic signals are present in these genes that are mostly congruent with chloroplast and nuclear gene phylogenies, and contribute to the resolution of challenging problems in angiosperm phylogeny including origins of parasitic angiosperms and the phylogenetic position of key basal lineages. Several noteworthy phenomena were observed, including horizontal capture of host plant DNA by parasitic plants and evidence of a chimeric atp1 gene containing a portion of a local host plant atp1 gene. Abrupt reversals of the editing status (Riveting) of all editing sites has

occurred repeatedly, implying frequent retroprocessing and recombination events involving edited mitotranscripts. These processes could drive high rates of correlated sequence evolution at editing sites, creating processed paralogs and destabilizing traditional concepts of homology. The impact of RNA editing on phylogenetic analysis is examined to revisit predictions made in an earlier study.

12.4.4. Mitochondrial sequences in monocot phylogenetics: new data, new problems

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Phylogenetic analyses of the Monocotyledons are based on larger and larger data sets including nucleotide sequence data from all three genome compartments. Incongruence between data sets from the three different genomes helps identify taxa that show anomalous behavior. Analyses of mitochondrial sequences from *cob* and *atp1* in all monocot families point at a number of taxa with divergent sequences, which either placed them on exceptionally long branches or grouped them in peculiar phylogenetic positions. Several processes may explain the divergences including elevated substitution rates, transfer of genes from the mitochondria to the nucleus, and horizontal gene transfer. Whereas an elevated substitution rate in the mitochondrial genes may account for the odd observations in some achlorophyllous taxa (Triuridaceae, Burmanniaceae, Corsiaceae), observations in other groups, e.g., the Liliaceae and the Anthericaceae, most likely are due to transfer of mitochondrial genes to the nucleus.

12.4.5. High frequency of intracellular gene transfer in grasses and persistence of transcompartmentally duplicated and expressed genes.

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Two classes of genes, encoding ribosomal proteins and SDH subunits, are lost from the mitochondrial genome and functionally transferred to the nucleus at exceptionally high rates during angiosperm evolution. We focus on two of these genes in grasses, and by greatly increasing their sampling, show that the dynamics of gene transfer, loss and persistence are even more complex than previously recognized. We show that *rps14* was functionally transferred to the nucleus - within the intron of the *sdh2* gene - early in grass evolution. Remarkably, a transcribed and RNA-edited *rps14* pseudogene has been pervasively retained in the mitochondrion for over 50 million years of grass evolution. The *rpl5* gene, usually separated from *rps14* by a single nucleotide, was transferred to the nucleus a minimum of five times in grasses, with a distinct gain of targeting sequences each time, and with retention and expression in many mitochondrial lineages. Our results show that two mitochondrial genes of close proximity can experience different fates of intracellular transfer and that the frequency of functional gene transfer can be even higher than previously estimated.

12.4.6. Exceptionally high substitution rates in Geraniaceae and Plantaginaceae mitochondrial DNA nad exon regions

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Nucleotide substitution rates in mitochondrial DNA of Geraniaceae and Plantaginaceae were found to be exceptionally high as compared with other angiosperm mtDNA lineages by Palmer and co-workers in 2000. Rates in *nad1* *b* and *c* exons were found to be high enough to distinguish species groups in *Pelargonium* by Bakker & al. in 2000. Palmer & al. had already shown that Geraniaceae and Plantaginaceae have lost their mtDNA introns. In this study we compile DNA sequence data from three exons of the mtDNA *nad1* gene for Geraniaceae (i.e. *Pelargonium*, *Erodium*, *Geranium* and the outgroup *Hypseocharis*) and Plantaginaceae (*Plantago* and its sister group *Limosella*) in order to assess the extent and nature of the nucleotide substitution rate acceleration. We also include further outgroups in order to establish where the increase in substitution rate has occurred. Comparison is made with plastid and, if possible, nuclear DNA sequence data in order to show that the rate acceleration is exclusive to mtDNA.

Furthermore we compare our findings with mtDNA *nad* sequence data from other angiosperm groups for which rate accelerations have not been reported (Burmanniaceae, Orchidaceae).

12.4.7. Utility of mitochondrial DNA in plant phylogenetic reconstruction

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Plant mitochondrial DNA, in comparison to plastid DNA, exhibits two unique features: a high frequency of structural changes and a low substitution rate. They both can be exploited to resolve difficult problems in the land plant phylogeny. We present phylogenetic analyses of basal angiosperms using sequences of mitochondrial genes *atp1* and *matR* and plastid genes *atpB*, *matK*, *rbcl*, and *rpoC2*. We evaluate relative contribution of the mitochondrial and plastid genes in resolving various nodes of the basal angiosperm phylogeny. We also present an investigation of distribution of group II introns in the mitochondrial genome of basal land plants as well as a phylogenetic analysis of group II introns in prokaryotes and organellar genomes of eukaryotes. These analyses help to assess informativeness of the genomic structural characters when used to resolve controversial issues in plant phylogeny, and provide an assessment of confidence level of the phylogenetic resolution.

12.5.1. Migration and evolution of arctic plants in response to Quaternary climate changes

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The modern Arctic is a comparatively young biome, formed in response to global cooling in the late Tertiary. The early Quaternary flora was probably recruited from survivors from the arcto-Tertiary forests combined with immigrants from various southern mountain ranges. This floristic mixture has since been repeatedly spatially rearranged and re-mixed in response to numerous cycles of warming and cooling. Here we address recent developments in deciphering and understanding the evolutionary and biogeographic history of arctic and arctic-alpine plants at various scales in space and time. We will summarize recent and ongoing studies at the entire circumpolar (and bipolar) to regional scales. These studies aim to infer origins of arctic plant groups from genus- or section-level phylogenetic and biogeographic analyses, origins of polyploid species complexes from systematic and phylogenetic analyses of markers such as low-copy nuclear genes to unmask their diploid progenitors, and origins of modern single-species distributions formed by postglacial expansion from their most recent glacial refugia, based on phylogeographic analyses of various markers.

12.5.2. Glacial refugia and directions of migrations of arctic alpine plants in Eurasia

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During the Quaternary large areas in the Arctic were repeatedly and heavily glaciated. Populations of arctic plants must have survived in unglaciated northern refugia and/or in more southerly mountain ranges, most likely in areas that provided suitable habitats all the way through the severe and rapid climatic fluctuations. Palaeoenvironmental and biogeographical data suggest that glacial refugia existed not only in Beringia, but also in other regions, such as in northwestern Siberia. We present phylogeographic/phylogenetic case studies based on cpDNA sequences and nuclear AFLPs markers for *Eritrichium* sect. *Eueritrichium* (Boraginaceae), *Thalictrum alpinum* (Ranunculaceae), *Pedicularis oederi* and related taxa (Scrophulariaceae), and the *Saxifraga stellaris* group (Saxifragaceae). The molecular data, combined with palaeoenvironmental evidence for potential refugia in Eurasia, are used to infer in which parts of northern Eurasia refugia were realized. These refugia may have been of general importance for long-term maintenance of arctic-alpine biota.

12.5.3. Immigration history of rare arctic-alpine plants into the Alps

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A considerable number of plant species of predominantly arctic distribution grow in non-arctic mountain ranges as well, a distribution type referred to as arctic-alpine. There are large differences between such taxa regarding the extent of their distribution area outside the Arctic. Whereas some arctic-alpine species occur frequently in many mountain ranges of the northern hemisphere, others are very rare, sometimes with only a few populations outside the Arctic. Here we address the immigration history of five rare arctic-alpine model taxa (*Carex atrofusca*, *Carex bigelowii*, *Juncus biglumis*, *Minuartia biflora*, *Ranunculus pygmaeus*) into the European Alps using AFLP fingerprinting and DNA sequencing. We focus on the circumpolar phylogeographical patterns, the source areas for the colonisation of the Alps, the mode of origin of the alpine populations (single vs. multiple introductions) and the phylogeographical structure within the Alps. Some of the rare arctic-alpine plants are critically endangered in the Alps, and conservation strategies will be discussed.

12.5.4. Reticulate evolution and phylogeography in the Arctic: *Saxifraga* section *Mesogyne*

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Saxifraga section *Mesogyne* is a small, well-defined group of mainly arctic-alpine plant species and is represented in most mountains and arctic regions of the northern hemisphere. We use various molecular markers and flow cytometry to study the origin, evolution, and migration of these saxifrages in relation to the Pleistocene glaciations. We analyse sequences from five non-coding chloroplast DNA regions and 1-2 nuclear DNA regions in addition to AFLP fingerprints. Preliminary phylogenetic analyses indicate that the section is monophyletic, but the origin of the section is not yet resolved. Several chloroplast haplotypes have wide circumpolar distributions, suggesting extensive migration. Most chloroplast haplotypes are species specific, but one widespread haplotype is represented in three species. Analyses of the nuclear sequences suggest that this is the result of hybridization and allopolyploidy, and that at least five species in *Mesogyne* probably have been formed this way. Thus, large-scale migrations and extensive reticulations seem to have dominated the history of *Saxifraga* section *Mesogyne*.

12.5.5. Comparative worldwide phylogeography of the genus *Arabidopsis* based on plastid and nuclear DNA markers.

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Modern plant systematics has shown that a circumscription of the genus *Arabidopsis* comprises the former genus *Cardaminopsis* and *Arabidopsis thaliana* only. "*Cardaminopsis*" as treated traditionally comprises three species groups: *A. lyrata*, *A. arenosa*, *A. halleri*. All of them are represented by different subspecies and/or cytotypes. Additionally, several taxa are of hybrid origin such as *A. suecica* and, as shown here, *A. croatica* and *A. cebnensis*. DNA sequence data of nuclear encoded ITS substantiate the existence of these three groups. However, cpDNA haplotype variation based on more than 1,000 samples revealed 5.5% shared haplotypes between species indicating higher levels of interspecific gene flow. Several phylogeographic studies of *A. thaliana* have been introduced within the last few years. Herein we present the first comparative northern hemispheric phylogeographic framework of the several "*Cardaminopsis*" species. We characterized high levels of genetic differentiation with more than 140 cpDNA haplotypes. However, pairwise genetic distances are low, indicating pleistocenic migration and in some cases periglacial survival.

12.5.6. Hybridization and adaptive radiation in Australian alpine *Ranunculus*

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DNA sequence data indicate late Pleistocene dispersal of *Ranunculus* (sect. *Acris*) from New Guinea to the Australian Alps followed by rapid ecological speciation and morphological divergence. The 8 Australian alpine species hybridize extensively.

Hybrid zones are narrow (often < 1m wide), with corresponding clines in morphological transition and allele frequency across ecotones. The species are fully reproductively compatible, with F₁, F₂ and BC hybrids vigorous and fertile. A three-year field trial of parental and hybrid seedlings indicates that hybrid zones are stabilised by intense ecological selection: species perform best in their own habitats; hybrids out-perform parental species within hybrid zones. Adaptive aspects of leaf shape inheritance were investigated using parental lines and hybrid (F₁, F₂ and BC) progeny arrays. Quantitative genetic models revealed polygenic control of traits by independently segregating genes. We propose that adaptations to exploit divergent habitats have acted to isolate lineages via intense natural selection. This study emphasises the importance of selection versus drift for speciation in insular habitats.

12.5.7. North American phylogeography

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During the Pleistocene epoch, when ice covered large portions of Eurasia, ice also buried much of northern North America. As in Eurasia, ecosystems were greatly shaped by the presence and absence of ice. Molecular phylogeographic methods reveal the presence of refugia north and south of the limits of North American ice sheets. Arctic and alpine habitats are highly disjunct on the continent, often forming islands of cold-tolerant species that are either closely related or conspecific, and were likely more widespread at the height of the Pleistocene. Ancient vicariance is also responsible for genetic discontinuities on the North American landscape. Such isolation among plant and animal populations, especially associated with orogeny, usually resulted in higher levels of genetic divergence than that derived from glacial isolation. Molecular tools can also provide the resolution needed to track post-glacial migration patterns and suture zones. The latter are important because they are communities of migrants brought together by different migration waves. The phylogeography of North America has many similarities to that of Eurasia.

12.6.1. Mating systems and speciation in lichen-forming ascomycetes

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Speciation processes are poorly understood in lichen-forming fungi. Our team focuses on Teloschistaceae. Phylogenetic analyses of mycobionts (ITS 1 + 2, 5.8S rDNA, hydrophobin, tubulin and mitochondrial genes; C. Eichenberger) and their *Trebouxia* photobionts (ITS 1 + 2, 5.8S rDNA, rbcL gene; S. Nyati) are combined with studies on mating systems in the fungal partner. Changes in reproductive strategies, from cross- to self-fertilization (hetero- to homothallism) or even loss of sexual reproduction, have a strong impact on speciation processes. As mating type genes evolve very rapidly they are difficult to analyse. Sandra Scherrer characterized the mating type genes of a range of *Xanthoria* spp. The distribution of *MAT* haplotypes in natural populations of heterothallic species was explored and the genetics of homothallism in two ubiquitous species investigated. One of these self-fertile species had both idiomorphs (*MAT 1-1* + *MAT 1-2*) per haplotype, the other a HMG box (*MAT 1-2*) only, an exceptional situation among ascomycetes. Genetic diversity within populations of homo- and heterothallic *Xanthoria* spp. and their photobiont was analysed (RAPD-PCR, rDNA).

12.6.2. Migration between continents: Geographic structure in *Porpidia flavocoerulescens* and *Porpidia melinodes*

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The influence of geography on the structuring of populations of the "species pair" *Porpidia flavocoerulescens* and *P. melinodes* was investigated. The extent of population subdivision was analyzed for populations located in North America, Greenland and Europe using analyses of molecular variance and F_{st}-statistics. Migration rates between sampling localities were estimated using MCMC-based importance sampling approaches to parameter inference. No clear population subdivision was observed. Haplotypes were shared between continents and geographic regions. The influence of geography on the populations could be best described by a

model of isolation-by-distance. The studied epilithic crustose lichens seem to have effective dispersal strategies. The type of dispersed propagules (i.e. ascospores or soredia) does not have a strong influence on the dispersal ability.

12.6.3. Two Group I introns in the 1516 and 1506 positions show a consistent geographical pattern in the *Parmeliaceae*

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We have studied the primary and secondary structure of 150 Group I introns of nuclear SSU rDNA in more than 60 species of the lichen family *Parmeliaceae*. The species studied can be divided into two groups, one showing an intron at the 1506 site and the other at the 1516 position. The two introns differ in sequence and structure and do not occur together. The 1516 introns observed are well conserved in other genera of Lecanorales, whereas the 1506 introns are not present in other Lecanoromycetes. 1506 introns showed significant correlations with a Group I intron at the LSU rRNA of one basidiomycete, with SSU rRNA introns of other ascomycetes and with Group I introns inserted in the SSU rDNA of some chlorophyta species. We have found a distinct correlation between the intron position and the geographic distribution of the species examined. The 1516 introns were found in boreal or temperate lichen species of the small genera *Pseudephebe*, *Asahinea*, *Parmeliopsis*, *Vulpicida*, *Arctoparmelia* as well as *Parmelia*, *Melanelixia*, etc. whereas the 1506 ones occur in species of the tropical genera *Hypotrachyna* or the oceanic temperate mid-latitude species of *Parmotrema* and *Flavoparmelia*.

12.6.4. Potential distribution modelling and niche characterization of Peltigerales order (lichens) in the Iberian Peninsula

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Most of the species included in the Peltigerales order are associated to well-preserved forests, and some of them are included in national and regional Red Lists. However, there is a lack of regional assessments of habitat suitability that identifies limiting ecological factors for the species. In this work we present a regional model for the distribution of 45 taxa of this order in the Iberian Peninsula. The occurrence in 100 km² UTM squares were modelled with broad-scale explanatory variables obtained from thematic digital cartography, climatic data and spatial coordinates. All data were integrated in a GIS tool. These models are relevant in the conservation of the species, because they suggest the limiting factors for these species in the Iberian Peninsula and, they generate predictive maps pointing out both areas in which conservation problems may be probably (suitable areas that are unoccupied), and areas where no data is available but these species are likely to be present. Furthermore, these models can be used as predictive tools in new warming climate scenarios to counteracting measures.

12.6.5. The *Bacidia rosella*-group in the Mediterranean Region.

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The species concept applied to study *Bacidia* De Not. has been based on central and northern European samples. The revision of Mediterranean material is providing a new outlook on the conception of several species, especially on *Bacidia rubella* (Hoffm.) A.Massal. and *B. rosella* (Pers.) De Not.

Former studies have placed most of the samples with orange to red apothecia and long acicular ascospores in *Bacidia rubella*, while those samples with a pinkish reddish apothecia and longer ascospores used to be included in *B. rosella*. Recently, *B. fraxinea* Lönnr. has been reinstated on the basis of thallus morphology and ascospores size. Afterwards, two new species have been described, *B. parathalassica* Llop & Gómez-Bolea and *B. iberica* Aragón & Martínez. Both species are segregated according to their thallus morphology, apothecial features and ascospore size. Molecular data from the recently described data is not available in order to substantiate its identity; however, morphological characteristics are consistent enough to maintain both taxa.

12.6.6. Unexpected genetic diversification in morphological uniform taxa and exceptional morphological diversification in *Cladia* and closely related lineages.

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Cladia is a genus of lichenized fungi with most of its species endemic to the Australasian region. In our phylogenetic analysis of partial nuclear and mitochondrial rDNA, the species of the genus *Cladia* were divided into two clades. The first clade, containing *C. aggregata*, the type species of the genus, consisted exclusively of the highly similar and hard to distinguish members of the *C. aggregata* complex. Despite of their morphological uniformity this clade showed unexpected high rates of genetic variation. The opposite observation was made on the second clade. It showed the regularly perforated species of *Cladia*, such as *C. retipora* and *C. sullivanii* paraphyletic relative to species of *Heterodea*, *Notocladonia* and *Thysanothecium*, three lichen genera occurring in the same geographical region. These three genera share many anatomical features with *Cladia* in general, but have very distinct vegetative morphologies. Their previous treatment as separated genera reflects the striking morphological evolution that has accompanied their diversification.

12.6.7. Towards a new taxonomy for the cetrarioid lichens (*Parmeliaceae*, lichenized ascomycetes)

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The monophyletic group of cetrarioid lichens is phylogenetically analysed using molecular data from three gene regions, nuclear ITS rDNA, betatubulin and GAPDH sequences, together with traditional structural characters and secondary chemistry. The genera, *Ahtiana*, *Allocetraria*, *Arctocetraria*, *Cetrariella*, *Flavocetraria*, *Tuckermanella* and *Vulpicida*, are nested within the three large cetrarioid genera *Cetraria*, *Nephromopsis* and *Tuckermannopsis*. A monophyletic clade of three *Tuckernaria* species, *T. ahtii*, *T. laureri* and *T. pseudocomplicata*, and 'Cetraria' *leucostigma* and 'C.' *melaloma* is nested within *Nephromopsis*. The status of the genus *Tuckernaria*, and other segregates within the group, is discussed and different alternatives to solve the taxonomy of the cetrarioid lichens are presented. A brief historical outline and a list of genera and species in current use is presented.

12.7.1. Dating the divergence of angiosperm radiations: progress and prospects

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Integrating these fossils into the tree of living angiosperm taxa remains essential for understanding both the origin of extant angiosperms and the origins of morphological features. Such attempts at integration have been rare for any group of organism; there has been surprisingly little communication between paleobotanists and systematists. We will explore the ways in which angiosperm fossils have been integrated into the phylogenetic framework with extant taxa, with the ultimate goal of forming a comprehensive phylogenetic tree of living and fossil angiosperms. We envision several components for discussion (theoretical and empirical) during this symposium, including: critical evaluation of the fossil record and appraisal of the age of fossils for major clades of angiosperms; construction of a morphological data matrix for fossils and extant angiosperms and the assessment of confidence in fossil placements; integration of fossils into the angiosperm tree with molecular data; exploration of the effects of missing data; and methodologies for the estimation of divergence times.

12.7.2. Dating the Marsileaceae: evolutionary and biogeographical implications

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Marsileaceae is a heterosporous fern clade of about 60 semi-aquatic species in three distinctive genera (*Marsilea*, *Regnellidium*, *Pilularia*). *Marsilea* is by far the most diverse genus (>50 species) and has a cosmopolitan distribution, yet species-level relationships

within the genus have been virtually unknown. Here, we present results from the phylogenetic analysis of a six-locus data set of coding and non-coding regions from the plastid and nuclear genomes of Marsileaceae. The fossil record has preserved a rich history of these ferns, mostly in the form of spore microfossils, indicating that Marsileaceae are relative newcomers that diversified during the Cretaceous (ca. 144 mya), at the same time as flowering plants. By integrating critical microfossils as age constraints, we are able to use our molecular data set to determine divergence time estimates for the intergeneric splits and for extant Marsileaceae species. Our phylogenetic hypothesis of extant taxa also serves as an explicit framework to reconstruct the biogeographical history of this group and its putative radiations in Africa and the Americas.

12.7.3. Integrating fossils into molecular phylogenies: examples from Early Cretaceous angiosperms

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Use of fossils to calibrate molecular divergences requires close attention to the concepts of crown group and stem lineage and cladistic analysis of morphology. Synapomorphies seen throughout a crown group provide only a minimum age for its stem lineage; synapomorphies of an extant subgroup are needed for a minimum age of the crown group. Fossils with both crown-group synapomorphies and more plesiomorphic states represent stem relatives; they do not indicate whether the crown group had evolved. Among Cretaceous relatives of Chloranthaceae, *Couperites* fruits with *Clavatipollenites* pollen may be nested within the family or stem relatives. Albian fruits with *Asteropollis* pollen provide evidence for the *Hedyosmum* line and a minimum age for the family, but crown-group *Hedyosmum* may be younger. Barremian-Aptian tetrad pollen can be linked with Winteraceae, but its finer sculpture indicates it is a stem relative. Barremian-Aptian tricolpate pollen may represent crown or stem eudicots, but Albian *Nelumbites* and *Sapindopsis* clearly belong to the crown group (Proteales). Because of its dense record, tricolpate pollen may provide a maximum age for crown eudicots.

12.7.4. Alternatives to the use of fossils for estimation of divergence times.

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The integration of fossil data into phylogenetic trees reconstructed using DNA sequence data from extant taxa is one way of estimating divergence times in these phylogenies. Many recent studies have employed this mode of calibrating phylogenies and have greatly enhanced our understanding of the timing of diversification in angiosperm lineages. However, this approach may be prone to error because of the difficulty in assigning fossils to nodes in the phylogeny and only providing minimum age estimates. Dates obtained by calibrating trees using fossils should ideally be corroborated with evidence from other sources. For some lineages the fossil record is so poor that there is no opportunity to utilize a fossil as an internal calibration point. Constraints on ages could also be based on tectonic events or dispersal of taxa to volcanic oceanic islands of known age. Alternatively, one could utilize the range of published substitution rates for a particular gene and apply these taking into account possible generation time effects. These alternatives are discussed with respect to examples in Rhamnaceae, Annonaceae and mimosoid legumes.

12.7.5. Patterns and rates of evolution in land plants: Fast vs. slow evolving genes.

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Understanding relationships among major clades of land plant provides a solid framework to evaluate rate of phylogenetic splitting and molecular evolution. Here, we examine the impact of mode and rate of gene evolution on phylogenetic reconstruction and estimation of lineage divergence times across land plants. A sample spanning all major extant land plant clades was examined using sequences of slow evolving (atpB, psaA, psbB, rbcL) and rapidly evolving (matK, trnL) plastid genomic regions. Using methods that account for molecular rate heterogeneity, we

evaluate effect of fast and slow genomic regions and different optimization criteria on phylogeny reconstruction and divergence time estimation, and compare rates of molecular evolution of these genomic regions across land plants. Our results show good congruence in phylogenetic relationships derived from fast and slow regions, and provide a general overview of the time of origin and diversification of major land plant clades.

12.7.6. Divergence times of phylogenetically basal eudicots

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A molecular dating of the phylogenetically basal eudicots (Ranunculales, Proteales, Sabiales, Buxales and Trochodendrales sensu APGII) has been performed using several fossils as minimum age constraints. We have sampled all rbcL sequences available in GenBank for the taxa in focus. Dating was done using Penalized Likelihood, and compared with NonParametric Rate Smoothing. We show that choice of method and fossil constraints has a great impact on the age estimates, and that it is important to use several fossil constraints to yield good age estimates. We discuss the 14 fossils we have chosen to include in this study and present a critical review of other fossils potentially useful in dating studies within the basal eudicots. Our results suggest a rapid diversification during the Early-mid Cretaceous, with all the lineages of basal eudicots emerging during the latest part of the Early Cretaceous. The age of Ranunculales was estimated to 120 myr, Proteales to 119 myr, Sabiales to 118 myr, Buxales to 117 myr and Trochodendrales to 116 myr.

12.7.7. Supertrees, supermatrices and a dated phylogeny of all palm genera

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The prevalence of palms (Arecaceae/Palmae) in the fossil record has resulted in the frequent assertion that the family is an ancient angiosperm lineage. This perception is, in part, skewed by the fact that palms are especially likely to be successfully fossilised due to the toughness of their organs and has never been explicitly challenged, nor have the ages of the major lineages within the palm family been explored within a solid framework. We have built the first complete phylogenetic tree of all 187 genera of palms using both supertree and supermatrix approaches. Having optimised *rbcL* sequence data from all palm genera across the tree, we have made branches proportional to time using non-parametric rate smoothing and have calibrated the tree with well-authenticated fossils. We use this tree to determine whether or not palms are truly 'ancient' and to examine the time frames in which the major radiations of extant palms occurred. By comparing the dated tree with tectonic and palaeoclimatic evidence, we evaluate the constraints and opportunities that may have determined the diversification of the family.

12.8.1. Using phylogenies to realign taxa in an emerging new classification of Leguminosae.

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The Leguminosae currently comprises 727 genera in 36 tribes. A decade of molecular phylogenetic study by several research groups around the globe has led to a substantial advance in the higher level systematics of the legumes and to the construction of a supertree of the family. Many genera and suprageneric groups, as traditionally circumscribed, are not monophyletic. Realignments of a significant number of these are, however, now strongly supported by the accumulating data. Legumes of the World (Lewis, Schrire, Mackinder & Lock, in press) is an illustrated encyclopaedic account of all legume genera placed in a phylogenetic framework. The volume uses published phylogenies to support taxonomic realignments that update the classification of Polhill (1994) presented in the Phytochemical Dictionary of the Leguminosae, and presents hypotheses of relationship for further testing. It also paves the way towards a radical new legume classification.

Examples of the major repositionings of taxa based upon recent phylogenetic research will be presented, together with the implications for the longer term restructuring of the whole family: from genus to subfamily.

12.8.2. Early floral development in Papilionoideae and its phylogenetic interpretation

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Early floral development of Papilionoideae was studied using scanning electron microscopy, and showed that organ initiation is more diverse than thought until now. While previous studies suggest that floral organs arise uniformly unidirectional from the abaxial towards the adaxial side of the flower, recent observations show variability in this respect. Besides unidirectionality, organ initiation was found to be either sequential, simultaneous, 'modified unidirectional' or bidirectional. Other outstanding features in the papilionoid floral development are initiated but early suppressed bracteoles, and the asymmetric androecium, in which the adaxial antepetalous stamen is formed out of the median plane. While the distinct asymmetry of mature flowers of some Papilionoideae (e.g., *Vigna caracalla*) is a character of the late floral development, androecial asymmetry is already found in early developmental stages. Using molecular phylogenies, I present a first attempt of a phylogenetic interpretation of the different modes of organ initiation as well as a discussion of the systematic relevance of early and late papilionoid asymmetry.

12.8.3. Phylogenetic analysis of floral ontogenetic and molecular characters in the Caesalpinioideae: insights into floral evolution in the basal Leguminosae

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The paraphyletic basal subfamily Caesalpinioideae comprises 161 genera and 3000 species, and includes some of the most diverse legumes in floral morphology. Recent molecular analyses suggest phylogenetic relationships that question previously held ideas about trends in legume flower evolution. We present a phylogenetic analysis of ~ 70 floral ontogenetic and morphological characters for over 50 representative species of caesalpinoid legumes. The floral characters are combined with *trnL*, *trnL-F* and *matK* cpDNA sequences. Resolution among major lineages is obtained mostly from the molecular data, but morphological characters help resolve relationships within some clades. Our analyses suggest that bracteole and hypanthium morphology at initiation and maturity are phylogenetically informative and thus under evolutionary constraint, while reduction in petal and stamen numbers occurs in parallel in several clades, suggesting evolutionary lability. Zygomorphy is expressed as a multitude of homoplasious morphs, providing conclusive evidence that the suite of characters that makes up zygomorphy cannot serve as indication of phylogenetic position.

12.8.4. The evolution of bird pollination in Australian pea-flowered legumes

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The papilionoid legumes appear to be ancestrally bee-pollinated and a specific trigger mechanism has been demonstrated in some. In Australia, native bees in several families have been observed as the main or only visitors to pea hosts. Bird-pollinated species are known in multiple genera and tribes and it appears that bird-pollination has arisen several times independently from bee-pollinated ancestors. Comparative analysis of molecular phylogenies will be used to test the hypothesis that bird-pollinated taxa are derived from bee-pollinated ancestors. We will also examine (1) whether the timing of pollinator switches is consistent with the availability of pollinators through the Cenozoic; (2) whether switches to bird-pollination have triggered radiations; (3) whether morphology is a reliable guide to pollination 'syndrome'; (4) whether independently evolved bird-pollination syndrome characters differ; and (5) whether bird-pollination is related to community type.

12.8.5. Evolutionary radiations in the Mimosoideae (Leguminosae)

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A phylogeny of the legume subfamily Mimosoideae was constructed using molecular data from four chloroplast gene regions (*ndhF*, *matK*, *trnL-F*, and *trnK*) and morphological characters. This phylogeny is used to examine patterns of evolution in the Mimosoideae. Based on rates of character change, there appear to be several rapid bursts of evolution, correlating with both morphological innovations and habitat shifts. The first radiation represents a shift from rainforest habitats to seasonally dry forests and is presumed to be relatively old as generic-level diversity is high. The second radiation is correlated with reintroduction to the tropical wet habitat as well as movement into southeast Asia. This second burst is presumed to be of more recent origin, exhibiting high levels of species diversity within a few large genera. A shift in chromosome number from $x=13$ to $x=14$ seems to be highly correlated with the first radiation event, as do morphological characters such as armature and habit.

12.8.6. Phylogeny, gene duplication, and polyploidy in legumes

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Polyploidy results in simultaneous apparent duplication of all genes, and thus shapes both individual gene families and whole genomes. Comparison of nuclear and chloroplast gene phylogenies provides reciprocal illumination: individual nuclear gene orthologies can be hypothesized, while the overall pattern provided by chloroplast genes can be corroborated with independent data. Orthologous nuclear genes can then be used to as a source of variation for phylogeny and for comparative mapping of divergent taxa, both of which are relevant for understanding the evolution of polyploidy. The existence of many pairs of homoeologous genes with similar divergence values produces a genomic signature for inferring polyploid events unrecognized from chromosome number alone. Gene family phylogenies can be used to test whether ancient polyploid events were shared among taxa such as *Glycine* and *Medicago*, whose common ancestor was the progenitor of about half of all papilionoid diversity. Phylogenies of nuclear and chloroplast genes within these two genera are keys to understanding patterns of allopolyploidy and autopolyploidy, respectively.

12.8.7. Newly recognised succulent biome: key to the origin and global distribution of Leguminosae?

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Four global biomes predictive of legume distributions are delimited. These include a newly recognised Succulent Biome. A supertree of Leguminosae constructed from existing molecular phylogenies, is converted to a taxon-biome tree by superimposing the biome affinities of c. 730 legume genera. Vicariant analyses are then used to detect a generalised pattern of biome relationships for legumes. A Tertiary Laurasian, rather than Cretaceous Gondwana, hypothesis for the origin of Legumes is supported both phylogenetically and by fossil evidence, and this is consistent with a Tethys Seaway-wide ancestral distribution, associated with tropical seasonally dry forest. A dry origin for legumes is in accordance with key morphological synapomorphies, a high nitrogen metabolism and the underpinning of the Succulent Biome diagnosing the major nodes in the phylogeny. Tropical wet Rainforest Biome taxa are now shown to be primarily derived from dry biome taxa. The ages of a range of trans-continental legume crown clades show a largely Neogene to Quaternary bias, supporting dispersal rather than vicariance as a predominant explanation for legume distributions.

12.9.1. 30 years of studies in the family Piperaceae

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Here I explored the taxonomic history of the Piperaceae (black pepper family), starting with F.W. Miquel's *Systema Piperacearum* in 1843-1844 through C. de Candolle posthumous key in 1923, and W. Trelease & G. T. Yuncker's monographic treatments for the Neotropics between 1950-1970. Contrasting views on classificatory schemes for Piperaceae proposed by those authors are examined for *Piper* and *Peperomia*, in particular for Paleotropical species of the former genus. A brief account of the importance of sampling, and adequate collections in *Piper* is provided and a preliminary scheme of classification is proposed for the genus, which takes into account recent phylogenetic analysis, but also data from floral structure and development. Finally I explore the use of tree architecture, seed coat structure, and stem anatomy, characters until now rarely used in *Piper* for solving the taxonomy of species complex.

12.9.2. Molecular evolution of the matK/trnK region (cpDNA) and evolution of Piperales

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Piperales (about 3500 species) is one of the largest orders within basal angiosperms. The matK gene and the non-coding trnK intron of some 100 species within Piperales and several outgroups have been sequenced. All families (except Hydnoraceae) and all genera (except Euglypha) are represented. The analyses resulted in maximal support for all major nodes and new insights into the relationships within Piperales. Parsimony and Bayesian inference are highly congruent. The matK/trnK region is widely known for its good phylogenetic signal and its wide range of utility in reconstructing relationships. In most cases, compared to other common regions, this is based on different evolutionary processes on the molecular level. A wide range of micro structural changes (microsatellites, inversions, frame shift mutations and indel events) is discussed as well as the utility in phylogenetics.

12.9.3. Hydnoraceae and its systematic affinities with Piperales

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In a previous investigation, the holoparasitic Hydnoraceae clade (Hydnora, Prosopanche) was placed within Piperales; however, its exact affinity remained unresolved. More recently, a data set of 6 genes (nuclear, chloroplast, mitochondrial) for three outgroup and 16 Piperales taxa was constructed. Branch and bound analysis gave one tree with relationships fully resolved for Piperaceae (5 genera) and Saururaceae (4 genera). In contrast, the Aristolochiaceae (4 genera), Lactoridaceae (Lactoris) and Hydnoraceae clades remained unresolved, even when faster-rate parasites were removed. Aristolochiaceae is composed of two strongly supported clades: Asarum plus Saruma (Asaroideae) and Aristolochia plus Thottea (Aristolochioideae). A sister relationship between Hydnoraceae and the latter received moderate bootstrap support. Morphological divergence and branch lengths on molecular trees indicate a comparatively high degree of evolutionary change in this group, thus we recognize four clades (families): Aristolochiaceae s. str., Hydnoraceae, Lactoridaceae, and Asaraceae.

12.9.4. Phylogenetic relationships of Aristolochiaceae subfamily Aristolochioideae based on molecular and morphological data.

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Recent morphological and molecular studies revealed that the subfamily Aristolochioideae (Aristolochiaceae) has a much more complex internal structure than that suggested by traditional systematic treatments. Based on a representative sampling, a combined molecular and morphological dataset was analysed to clarify the phylogenetic relationships. *Thottea*, a small genus of Asian shrubs branches first, followed by *Isotrema* occurring in Asia as well as North and Central America. The next clade, *Pararistolochia*, from tropical rain forests of Africa and New Guinea, is sister to an extended genus *Aristolochia*. The latter

contains two monophyletic clades: pentandrous species from Central America (*Einomeia*) and a group representing mediterranean and paleotropical species (*Aristolochia* s. str.). Their phylogenetic position will be discussed with respect to paraphyletic Central- and South American hexandrous species.

12.9.5. Studies in Peperomia (Piperaceae): insights from molecular and morphological approaches

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With approximately 1500 valid species (3000 described), the genus *Peperomia* is one of the largest genera of the Angiosperms. As a result of this huge number of taxa and because of the difficult synonymy, the actual taxonomic knowledge of the genus is quite chaotic. The current infrageneric classification includes some 400 species and is primarily based on fruit morphology. Most species descriptions after 1900 are written without reference to their subgeneric affiliation. Additionally, the monophyly of the infrageneric groups has never been tested.

The overall aims of our work are: 1) to gain insight into the infrageneric relationships based on molecular data from two genomes and morphology; 2) to produce an interactive key to the species via WWW; 3) to revise the genus *Peperomia*. Preliminary results do support the current infrageneric classification on section level but not on subgenus level.

12.9.6. A Phylogenetic Analysis of Canellaceae based on Morphology and DNA Sequences

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Canellaceae is a small (22 species, 6 genera), tropical and subtropical plant family of aromatic trees (rarely shrubs) found in the Americas, eastern and southern Africa, and Madagascar. Canellaceae traditionally has been used for medicine and other purposes owing to their volatile oils. Most species of Canellaceae are threatened because they are locally or regionally rare, overexploited by humans, or their habitat has been destroyed. A phylogenetic analysis was conducted using morphological data and chloroplast gene sequences. Twenty-one species were sampled including several undescribed species from Brazil, Costa Rica and the Antilles. The results of this analysis indicate that Canellaceae is monophyletic and sister to Winteraceae. The monophyly of Canellaceae is supported by many characters including monadelphous stamens and parietal placentation. Within Canellaceae, *Pleodendron*, *Cinnamosma*, and *Warburgia* were resolved as monophyletic. However, *Cinnamodendron* was placed in two separate clades, representing a South American group with bicarpellate flowers and few ovules, and an Antillean clade with tetracarpellate flowers and many ovules.

12.10.1. The essential features of CO₂ concentrating mechanisms in plants.

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The inefficiencies of C₃ photosynthesis are largely due to the bifunctional enzyme Rubisco. Rubisco has a poor affinity for CO₂ and O₂ acts as a competitive substrate. The oxygenation reaction necessitates the recycling of phosphoglycolate with consequent loss of CO₂. Some photosynthetic organisms have developed ways to increase CO₂ concentration at the site of Rubisco, allowing the enzyme to operate closer to its maximal carboxylation rate and this also reduces oxygenation. The C₄ pathway, with its complex combination of biochemical and morphological specialisation, is an excellent example of such a CO₂ concentrating mechanism. A mathematical model of single cell C₄ photosynthesis with a focus on CO₂ diffusion is used here to explore the key parameters necessary to make the C₄ pathway function efficiently. Attempts are being made to introduce C₄ photosynthetic characteristics into C₃ crop plants by genetic manipulation. The modelling examines the theoretical efficiency of such a photosynthetic CO₂ pump, using available data on CO₂ diffusion characteristics of C₃ leaves.

12.10.2. Single-cell C₄ photosynthesis in *Hydrilla*: unraveling key components for a transgenic C₄ rice plant

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An understanding of the facultative C₄ system in the monocot *Hydrilla verticillata* can aid the development of transgenic C₄ rice plants because its C₄ and Calvin cycles operate in series in the same "C₃" cell, without Kranz anatomy. Cytosolic PEPC is segregated from rubisco and the decarboxylase NADP-ME in the chloroplasts, where CO₂ is concentrated. Multiple isoforms of PEPC and NADP-ME exist in *Hydrilla*, but *hvpepc4* and *hvme1* encode proteins with characteristics specific to C₄ photosynthesis. CA is also up-regulated in C₄ leaves, presumably in the cytosol to aid PEPC fixation, but we speculate it may be down-regulated in C₄ chloroplasts. Its role and sites(s) are under investigation. To avoid photoinhibition and maintain a favorable NADPH/NADP⁺ ratio in C₄ leaf granal chloroplasts we postulate OAA (possibly aspartate) is translocated and reduced to malate for decarboxylation. A search for differentially-regulated translocators is ongoing. A preliminary differential display identified about 60 differentially-regulated genes in C₄ *Hydrilla* leaves; 13 were confirmed by northern/RT-PCR analyses. Supported by USDA/CSREES/NRI No. 2002-35318-12540.

12.10.3. Single-cell C₄ photosynthesis in terrestrial plants

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Recently, existence of a unicellular C₄ photosynthesis mechanism was proven for terrestrial Chenopodiaceae species, *Borszczowia* and *Bienertia*, with two unique anatomical subtypes. In *Borszczowia*, organelles are partitioned towards the proximal and distal ends of elongated chlorenchyma cells which function analogous to mesophyll and bundle sheath cells, respectively, in Kranz type NAD-malic enzyme (ME) C₄ species. In *Bienertia*, the peripheral cytoplasm with chloroplasts is connected by cytoplasmic channels to a central cytoplasmic compartment in the center of the vacuole, with numerous chloroplasts and mitochondria which are shown to function analogous to mesophyll and bundle sheath cells, respectively, in classical NAD-ME type. Our results show two chloroplast types with dimorphism in structure, starch storage, and photosynthetic enzymes. This includes location of pyruvate, Pi dikinase in chloroplasts associated with capture of atmospheric CO₂, and compartmentation of chloroplasts containing Rubisco and ADPG pyrophosphorylase along with mitochondria containing NAD-ME and glycine decarboxylase, where C₄ acids donate CO₂ to the C₃ pathway.

12.10.4. Introducing C₄ photosynthesis into rice

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C₄ plants have a number of advantages, including high photosynthetic performance and high nitrogen- and water-use efficiencies. To transfer C₄ traits to C₃ plants, enzymes involved in the C₄ photosynthetic pathway were overproduced in the mesophyll cells of rice leaves. Each of four C₄ enzymes, namely, the maize phosphoenolpyruvate carboxylase (PEPC), the maize pyruvate, orthophosphate dikinase (PPDK), the maize NADP-malic enzyme (NADP-ME), and the sorghum NADP-malic dehydrogenase (NADP-MDH), were successfully expressed at high levels in transgenic rice leaves. Overproduction of any of PEPC, PPDK, and NADP-MDH did not significantly affect either photosynthesis or growth of rice plants, while that of NADP-ME led to serious stunting and leaf photobleaching. Such detrimental effects of the maize NADP-ME could not be overcome by co-overproduction of PEPC, PPDK, NADP-MDH, or a combination of the three. Thus, the rice NADP-ME was adopted instead of the maize enzyme. Quadruple transgenic rice plants had been produced and their homozygous lines are now being analyzed.

12.10.5. Expression of cyanobacterial *ictB* in higher plants enhanced photosynthesis and growth

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Under many environmental conditions plant photosynthesis and growth are limited by the availability of CO₂ at the site of RubisCO. We expressed *ictB*, a gene involved in HCO₃⁻ accumulation in *Synechococcus* PCC7942, in *Arabidopsis thaliana* and *Nicotiana tabacum*. The transgenic plants exhibited faster photosynthetic rates than the wild types (WTs) under limiting, but not under saturating CO₂ levels. Similar results were obtained in *Arabidopsis* plants bearing *ictB* from *Anabaena* PCC7120. Under low humidity, growth of transgenic *A. thaliana* plants was considerably faster than that of the WT. There was no difference in the amount of RubisCO or its activity (when activated *in vitro*) in the WT and the transgenic plants. In contrast, the *in vivo* RubisCO activity, without prior activation, was considerably higher; and the CO₂ compensation points was lower in *ictB*-expressing plants than in their WT. suggesting a higher CO₂ concentration in close proximity to RubisCO. This may explain the higher activation level of RubisCO and enhanced photosynthesis and growth in the transgenic plants. These data indicated a potential use of *ictB* for the stimulation of crop yield.

12.10.6. Glycolate oxidation in the chloroplast: A novel approach for the suppression of photorespiration.

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In C₃ plants, RUBISCO catalyses both the carboxylation and the oxygenation of Ribulose-1,5-bisphosphate. Carboxylation accounts for net CO₂ fixation whereas oxygenation leads to the loss of CO₂ in the photorespiratory pathway. CO₂ release in the mitochondria during photorespiration results in approximately 25 % loss of the carbon from phosphoglycolate. Moreover, NH₃ is lost in this cycle that has to be refixed. Our project aims to introduce a novel method for the partial suppression of photorespiration in C₃ plants. For this, a biochemical pathway from *E. coli* converting glycolate to glycerate was transferred to plant chloroplasts. In a first step, glycolate is oxidized to glyoxylate by a NAD(P)-dependent, but oxygen-independent Glycolate Dehydrogenase. Two molecules of glyoxylate are ligated to one molecule of tartronic semialdehyde (TS) under release of CO₂ that is immediately available for efficient refixation. TS is reduced to glycerate, a component that can be re-integrated into the plant basal metabolism. We will present data on the molecular and physiological characterisation of transgenic plants overexpressing the complete pathway.

12.10.7. Genetic manipulation of the photorespiratory C₂ cycle

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The photorespiratory C₂ cycle is indispensable for higher plants to survive in an oxygen containing environment. Moreover, the C₂ cycle represents the major source of internal CO₂ release in C₃ plants and, in course of early C₄ plant evolution, it presumably provided a primary CO₂ concentrating mechanism. The biochemistry and cellular biology of the metabolic processes that make up the C₂ cycle is very complex and, despite long-term research efforts, not fully understood. Its analysis is further complicated by the fact that most of the C₂ cycle enzymes are encoded by gene families but only some by single genes. We will present results from an analysis of *Arabidopsis thaliana* T-DNA insertional mutants that are blocked in individual enzymatic steps including, for example, glycine decarboxylase and hydroxypyruvate reductase. In light of these new data, we will discuss some open questions and recent developments in our understanding of the C₂ cycle.

12.11.1. The role of terrestrial non-vascular vegetation in biosphere-atmosphere exchange

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Huge areas of terrestrial surfaces are colonized by a microphytic covering, such as algae, cyanobacteria, bacteria, fungi, lichens and mosses, representing potential carbon sinks as they develop stable micro-vegetation canopies (biological crusts) if untouched. They often represent a dominant vegetation type in semi-arid environments with low primary production. Based on recent measurements in the Negev desert the contribution of biological soil crusts to the potential primary production ranges between 1-7 %. Emission of volatile organic compounds (VOC) between crusts

and the atmosphere may contribute to a carbon loss and as well influence atmospheric chemistry and physics. Some VOC species as well as the reduced sulfur compound carbonyl sulfide (COS) are also deposited and consumed. The talk will cover some aspects of the biosphere-atmosphere exchange of carbon, reduced sulfur compounds and volatile organic compounds with particular respect to soil crusts, lichens and mosses.

12.11.2. Carbon sequestrations by Larch Forest Ecosystems

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We routinely measure net ecosystem exchange (NEE) with eddy covariance method at the larch forests in northern Japan, northeastern China and central Siberia. We also partition component CO₂ fluxes, i.e., by utilizing a 24-automated-chamber system for canopy foliage photosynthesis (GPP) and respiration (R_i), a 24-automated-chamber system for stem and branch respiration (R_w), and a 24-automated-chamber system for the understory carbon budget (i.e., 8 chambers for soil efflux, R_s; 8 chambers for heterotrophic respiration, R_h; and 8 chambers for vegetation photosynthesis, R_v). For a 50-year-old larch forest in Japan, three year (2001-2003) averaged NEE was -186 and -500 g C m⁻² yr⁻¹, ecosystem respiration (R_e) was 1270 and 1095 g C m⁻² yr⁻¹ measured by closed-path and open-path eddy system, respectively. With the chamber approaches, GPP, NPP (net primary production), NEP (net ecosystem production) and R_s was estimated to be about 1275, 677, 130 and 960 g C m⁻² yr⁻¹, respectively. R_i, R_w, Root respiration (R_r) and R_h was 110, 75, 413 and 547 g C m⁻² yr⁻¹, which contributed 8%, 6%, 32% and 43% of GPP, respectively.

12.11.3. In-situ emission measurements of volatile organic compounds (VOC) from biological soil crusts (BSC)

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Volatile organic compounds (VOC) emissions from biological soil crusts (BSC) were investigated in the Negev desert, Israel, using dynamic cuvettes on permanent soil borne collars. Air samples from BSC/soil-enclosures were collected on carbo-cartridges. A gas chromatograph with flame ionisation detection (GC-FID) was used to analyse the samples for more than 20 VOC species including alkanes, isoprene, and terpenoids. Within most sample sequences the amounts of VOC were very variable or not different from reference measurements. However, some samples sequences with BSC showed significant emissions of VOC such as isoprene, octane, octanal, and 1-octen-3-ol. As a rough conclusion these first results of BSC's VOC exchange suggest that (1) their carbon loss due to VOC emission is mostly very low, and (2) significant VOC contributions to processes of biosphere-atmosphere exchange and atmospheric chemistry can be expected only for a limited part of their annual activity period.

12.11.4. Physiological analysis of response of mustard genotypes to sulphur nutrition

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Sixteen mustard [*Brassica juncea* (L.) Czern. & Coss.] genotypes were grown hydroponically for 35 days in nutrient solution supplemented with 5 µM and 20 µM S, and analyzed for S use efficiency (SUE), plant S concentration and content, and the distribution of S between old and young parts of the shoot. There were marked differences in SUE among mustard genotypes. Pusa Bold was about 2-fold and 7-fold more S-efficient than Pusa Jai Kisan in whole shoot and young-part based SUE, respectively. Interestingly, the older portions of the shoot for most genotypes had higher dry matter production under S deficiency than under sufficient S supply, suggesting that there was a significant inhibition of new shoot growth and transport of photosynthates

from source to sink organs under low-S conditions. Sulphur concentrations of both old and young portions of the shoot did not correlate with SUE, but shoot S content was found to be significantly correlated with SUE. Furthermore, S-efficient genotypes distributed more S into young parts of the shoot under S-deficient conditions than did the inefficient lines.

12.11.5. The leaf water uptake role in the water economy of Amazonian tropical trees.

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Early studies have shown the importance of dew absorption by leaves in regions where fog is an important component. However, the importance of water uptake by leaves of trees in tropical forests has not been previously considered. We hypothesize here that leaf water absorption in tropical trees might be responsible for the drought tolerance exhibited by Amazonian forest trees. Application of deuterium (D) labeled water and leaf water potential (Ψ) measurements were carried out in 3 common primary forest tree species at Flona Tapajós (Pará State-Brazil): *Coussarea racemosa*, *Miconia egensis* and *Eschweilera pedicellata* to test whether their leaves or stems absorb water directly. Two leaf irrigation treatments were carried out: leaves were sprayed with D water and branches were exposed to D water (1m from tip) with a moist cotton band. The leaf water D abundance in the sprayed leaves were significantly above background for all 3 species and followed the water potential response with species having the greatest response also having the greatest D abundance. These results confirm the role of water absorption by leaves in the recovery of water-stressed plants.

12.11.6. Long-term effects of short-term soil amendments in a sub-alpine grassland

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Many experiments in grasslands have shown that soil amendments such as fertilization can strongly affect vegetation and microbial composition, but there is a lack of long-term studies assessing over which period ecosystems will be able to recover, once the amendments have ceased. We studied in a controlled, large-scale field experiment in a subalpine pasture in the Swiss Alps the long-term effects of 2-4 years of NPK-fertilization and liming on vegetation and soil microbial communities. Nearly 70 years after the last application, liming still significantly affected vegetation composition and microbial community. The concentration of Ca-ions and pH was higher in limed plots, which was reflected by higher abundances of indicator plant species and PLFAs for low pH in the unlimed plots. We therefore propose that the observed changes in above- and below-ground communities were mediated through changes in soil pH. Our findings suggest that subalpine grasslands are highly sensitive to even short periods of disturbances, and that the recovery rate of certain ecosystem variables can be very low.

12.12.1. The terpene synthase gene family: Tools for ecological and evolutionary studies and metabolic engineering

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The remarkable abundance and diversity of terpenoid metabolites in plants and their increasing commercial importance as flavors, fragrances, pharmaceuticals and agricultural compounds have stimulated research on this enormous group of phytochemicals. The incredible skeletal diversity of terpenes is largely due to a major group of enzymes known as the terpene synthases. We are employing genetic and genomic tools to study the terpene synthase genes of *Arabidopsis*, maize and other species. Sequence comparisons, functional characterization and gene

expression studies along with profiling terpene metabolites have provided new information about the ecology, evolution and distribution of terpenes in these species. One striking conclusion is that, although monoterpene (C₁₀) and sesquiterpene (C₁₅) formation in plants was once thought to be associated solely with specialized oil cells, ducts, trichomes or secretory cavities, low levels of these compounds are probably universally emitted in the headspace.

12.12.2. Gain and loss of fruit volatile terpenoids produced by wild and cultivated strawberry species

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The molecular mechanisms that fruit flavors are gained and lost during evolution and domestication are largely unknown. While the terpenoid profile of cultivated strawberry species is dominated by linalool and nerolidol, fruit of wild strawberry species emit mainly carvyl acetate and myrtenol which are not detected in the cultivated species. Through cDNA microarrays and PCR we cloned genes responsible for the biosynthesis of these terpenoids. Detailed characterization of the different terpene synthases unraveled molecular evolutionary mechanisms which might explain the differences in the volatile terpene profile between wild and cultivated strawberry species. The loss of one metabolite further influenced the fruit flavor as it was no longer available as substrate for the downstream production. This was demonstrated by cloning and characterization of a cytochrome P450 gene, encoding the enzyme catalyzing the C10 hydroxylation of α -pinene to myrtenol, a typical wild strawberry compound.

12.12.3. Characterization of geraniol synthase and geraniol dehydrogenase, the enzymes responsible for the formation of citral in the glands of sweet basil

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The monoterpene fraction of the lemon-scented sweet basil (*Ocimum basilicum*) cv. Sweet Dani consists mostly of citral (a mixture of geraniol and neral), with lower levels of geraniol and nerol. These compounds are stored in glands found on the leaf epidermis. Geraniol synthase (GES) activity, generating geraniol from geranyl diphosphate, was shown to be localized exclusively or almost exclusively to glands. A GES cDNA was isolated based on analysis of a glandular trichome EST database, and the sequence of the protein encoded by this cDNA shows some similarity to sequences of other terpene synthases. We have also isolated a basil cDNA encoding NADP-dependent geraniol dehydrogenase (GEDH) and characterized the enzyme it encodes. GEDH is capable of oxidizing geraniol and nerol in equal efficiency. Prolonged incubation of geraniol with GEDH *in vitro* produces not only geraniol and its *cis*-isomer neral, but also nerol. The reduction of neral (which is likely produced by keto-enol tautomerization of geraniol) by GEDH explains the small amounts of nerol found in basil glands, since no enzyme is known to catalyze the formation of nerol directly from geranyl diphosphate.

12.12.4. The terpene synthase family in *Zea mays* and its role in defense against herbivory

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Terpenes constitute the largest group of plant products and display an extreme chemical diversity. In an effort to understand the genetic and molecular basis of terpene diversity as well as the ecological function of the complex terpene blends, we started to study maize terpene biosynthesis. We identified a large and diverse gene family encoding terpene synthases which each form multiple terpene products from a prenyldiphosphate substrate. Comparison of the terpene synthase *in vitro* activities with the terpene blends released by maize plants indicates that five differentially regulated multiproduct terpene synthases are sufficient to produce the complex maize sesquiterpene hydrocarbon blends. The characterization of structure-function relationships between the terpene synthases TPS4 and TPS5 revealed that the formation of products with stereospecific differences is determined by only four amino acids. Further

mechanisms increasing the diversity of maize terpene biosynthesis are based on the allelic distribution of active and inactive enzymes in maize lines. We are currently evaluating the ecological role of specific terpenes in maize defense.

12.12.5. Unraveling the wizardry of terpene biosynthetic enzymes

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Many plants respond to pathogen attack by the synthesis and secretion of anti-microbial compounds. For example, solanaceous plants produce anti-microbial terpenes that inhibit germination and growth of several fungal species. The production of these chemicals has been interpreted as an important defense response. We have also hypothesized that an understanding of the mechanisms responsible for the biosynthesis of the anti-microbial terpenes should provide a means for engineering the generation of novel and more efficacious compounds. Towards that goal, we have elucidated a 2-step biosynthetic pathway for capsidiol, an anti-microbial sesquiterpene di-alcohol. The pathway consists of a synthase that catalyzes the cyclization of farnesyl diphosphate to a bi-cyclic hydrocarbon structure, followed by the action of a P450 hydroxylase that introduces hydroxyl functions with stereo- and regio- specificity. Using several different approaches, we have mapped functional features of the respective enzymes, and used this information to evolve novel catalytic activities for the generation of unique chemical entities.

12.12.6. cDNA isolation and functional expression of Linalool Synthase from *Nicotiana glauca*

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Flowers of *Nicotiana glauca* (Solanaceae) emit a broad range of volatile organic compounds (VOCs), comprising primarily mono- and sesquiterpenes and aromatic esters. Linalool, an acyclic monoterpene, is a major component emitted from *N. glauca* flowers during the night. A cDNA encoding a monoterpene synthase was isolated via RT-PCR from petals. The nucleotide sequence of this terpene synthase consists of 1629 bp encoding a protein of 542 amino acids. The protein sequence is similar to other monoterpene synthases from different plant families. The heterologous expression of the tps clone allowed purification and characterization of this enzyme. It converts geranyl diphosphate into linalool and is therefore designated Linalool Synthase (LIS), although the protein shows highest identity at the amino acid level to an α -terpineol synthase from *Vitis vinifera*. The *lis* gene is only expressed in petals and anthers, not in other flower and plant tissues. High transcript levels during night correlate with the nocturnal emission.

12.13.1. 'Green' fingerprints of climate change

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Climate is a major determinant for the phenology, physiology, distribution and interactions of plants. With the world's recent increase in global average temperature, these processes are supposed to be affected in a perceptible way. An ever increasing number of recently published studies strongly suggests that the last thirty years of warmer temperatures have had a substantial influence on seasonal patterns and the distribution of plants and vegetation types. Common features of such "green" fingerprints of climate change are presented and discussed in terms of its implications for community structure, trophic interactions and ecosystem consequences. Though climate impact research has made some progress, it still suffers from the lack of long time series of standardised observations. There is an urgent need to establish global networks for ground based ecological monitoring such as GLORIA, the Global Observation Research Initiative in Alpine environments.

12.13.2. global change and the bioclimatology of grasses pollination optimum

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Grasses are the most cosmopolitan allergophytes. A large part of aerobiological researches have been devoted to produce forecasts using regression analyses between daily airborne pollen counts and meteorological factors. However, in these studies, the bioclimatology and eco-physiology of Grasses pollination were forgotten. By studying the aerobiology of Grasses pollen in Montreal for the past 19 years we were able to show that both seasonal total and maximum daily value (/m³) presented a decreasing trend, which could be correlated to a similar trend for rain fall, putting some doubt to the popular belief that global change will induce more allergy symptoms. By drawing frequencies curves of pollen abundance for different meteorological condition categories, we were able to visualize Grasses pollen behaviour: with temperature, the profile was negatively asymmetrical, meaning that, in Montreal, there is always a deficit of temperature for Grasses pollen; for relative humidity and wind speed, the profile was symmetrical, meaning that both have specific optimum; while for rain, it was parabolic, both arms representing rainout and washout processes.

12.13.3. Changing vegetation patterns at the alpine-nival ecotone of the Eastern Alps - the last 10 years on Mount Schrankogel/Tyrol

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Comparisons of historic data with records from recent investigations provided evidence that vascular plant species richness has increased on high summits of the Alps during the 20th century. This upward migration of alpine plants was most likely caused by the observed climate warming since the 19th century. Stimulated by these findings, an extensive monitoring network of 1000 one-square metre permanent quadrats was established on Mount Schrankogel in the Tyrolean central Alps at altitudes between 2900 and 3450 m a.s.l. in 1994. In 2004, a representative selection of 400 of these 10-year-old plots were re-investigated. Some pioneer species of alpine grassland were remarkably expanding or have established at new sites, while others have disappeared or showed a decreased cover. The extent of changes appeared to be dependent on the topographic position and thus on the snow cover duration.

12.13.4. Climate change and its impact on cloud forests: Fire as a major determinant in the subalpine zone of Kilimanjaro

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The vanishing glaciers of Kilimanjaro attract broad interest. Less conspicuous but ecologically far more significant is the associated increase of frequency and intensity of fires on the slopes of Kilimanjaro, which lead to a downward shift of the upper forest line by several hundred meters as result of a drier (warmer) climate since the last century. During the last 70 years Kilimanjaro has lost nearly half of its forest cover, in the upper areas caused by fire, on the lower forest border mainly caused by clearing. The loss of 150 km² of cloud forest - the most effective source in the subalpine fog interception zone - caused by fire during the last three decades means a considerable reduction in water yield. In contrast to common belief, global warming does not necessarily cause upward migration of plants and animals. On Kilimanjaro the opposite trend is under way, with consequences more harmful than those due to the loss of the showy ice cap of Africa's highest mountain.

12.13.5. Plant and environment in the subnival belt of the Caucasus

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Subnival belt (2900-3700 m) is quite well formed in Georgia. It is distinguished by high degree of endemism (including generic endemism) compared with the Alps. It is also distinguished by participation of Caucasian-West Asian geographic elements. Plants are distributed sporadically in the subnival belt in a form of

separate individuals as well as nanocoenoses. The facilitation phenomenon is a feature of the belt, i.e. plants typical of the subnival belt shelter plants of other belts. Observations made with the support of the GLORIA-EUROPE project have shown that there are two tendencies of plant adaptations to severe winter conditions: plants protected by snow cover and plants without snow cover. We suggest that the latter must be connected in part with the global warming, considering that upper borders of distribution of certain species have already ascended by approximately 50-80m.

12.13.6. Climate change and its impacts: facts and fiction

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Much of current thinking about global warming and its environmental impacts is based on assumptions and computer models. These assumptions and model outputs are carried forward as facts into successive cycles of research through scientific publications and form the basis for training new generations of scientists. Whilst accepting that science is not about finding the truth but about testing hypotheses by the application of scientific methods, foremost logic, it is argued that uncritical acceptance of 'computer output reality' is counterproductive to progress. A critical overview is presented of evidence-based vs. assumption- and computer simulation-based research on the impacts of climate change (temperature warming, changing precipitation) and nutrient input from atmospheric deposition on alpine plants and ecosystems. Following a discussion of the characteristics of alpine habitats and the ecological principles governing species co-existence, conclusions from field observations, experiments, historical evidence and model predictions are compared.

12.13.7. Short-time effects of simulated climate change on epiphytic communities of tropical cloud forest

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In order to address how climate change might alter epiphyte communities in tropical cloud forests and if this response will take place at community or specific level, we transplanted complete epiphytic bryophyte communities in the Bolivian Andes. We moved 60 branches with epiphyte communities from 3000 m to 2700 m and 2500 m, simulating temperature increases of 1.5°C and 2.5°C. Controls were transplanted at 3000 m. On each branch we recorded the cover of each species at the start and after a year, and calculated Sorensen's similarity values comparing the composition across time. Communities moved to lower elevations changed faster than communities at their original elevation. At specific level, some species increased while others decreased. According to these results, it is possible to suggest that epiphyte communities may be already reacting to climate change and that future community composition will depend on the individual response of each species.

12.14.1. The past, present and future of botanic gardens

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The reasons for establishing and the primary objective of botanic gardens have evolved and changed over time: from early European university teaching and research gardens to economic trade focused gardens of the colonial era to today's diversity of missions and objectives. The last fifty years have been marked by a period of remarkable growth in the number, the role and the distribution of botanic gardens. The BGCI *Garden Search* contains online information on over 2,400 botanic gardens operating in 167 countries today.

The *International Agenda for Botanic Gardens in Conservation*, developed by BGCI and the botanic garden community, provides a global framework for the development of individual garden's strategies within a common goal of stemming the loss of biodiversity. It is the *International Agenda* and the *Global Strategy for Plant Conservation* that will help shape the future of botanic gardens. Through the GSPC, botanic gardens have an opportunity to play an important role in connecting work on biodiversity conservation to the issues of sustainable development, the

alleviation of poverty and achievement of the Millennium Development Goals.

12.14.2. The International Association Of Botanic Gardens

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Since its independent constitution in 1981, IABG has represented a basic structure with continental networks, promoting the European-Mediterranean association in Hamburg 1982, and the Asiatic one in Nanjing 1988. In the Nancy Conference (1984), IABG and IUCN did not find an effective mechanism for conservation cooperation, so IUCN promoted its BGCS, later the independent BGCI. In Wuxi, China (1993), BGCI and IABG signed an agreement to promote common initiatives, congresses and workshops. IABG did never try to organize a formal membership but is devoted to promote new networks. It always kept a message, including not only conservation but also social commitment and natural and cultural heritage. IABG and BGCI lead their actions towards this last line in the core of the EU, and created the European Consortium. In Barcelona 2004, IABG endorsed the Spanish network document "*Botanic Gardens: an increasing value*", and encouraged the UNESCO recognition of a World Day of Botanic Gardens.

12.14.3. The history, conservation challenges and future tasks of the Botanical Garden at National Museum of Natural Science, Taichung, Taiwan

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The Botanical Garden at National Museum of Natural Science carried out the design and the plan in March, 1994 and was opened to public in July (established by January,) 1999. It includes a 4.5-ha open field for the varied and representative native vegetations at the low altitude of Taiwan, a 33 m tall hexagonal climatron for displaying the tropical rainforest, an exhibition greenhouse for ferns or special floral exhibits, the nursery area for conservation as well as research, and the herbarium (TNM) for specimens. Due to the roles of this Garden containing recreation, conservation, education, exhibition, public service and research, it combines the great challenges to fulfill all the missions and achievements. In passing 5 years, over 1,800 species of growing plants, 200 species in seed banks, 20 special exhibits, 3 symposia, 100 educational events and achieving 110,000 specimens. The optical daily records exceeded 5000 visits per day for special exhibit in January 2005. The future perspectives crucially focus on the management of species diversity, recording phenology, conservancy projects, exhibit improvement and institutional cooperation.

12.14.4. Developing an action plan for Fushan Botanic Garden, Taiwan--- A dream for people who appreciate biodiversity

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The Fushan Botanic Garden (FBG), located in northeastern Taiwan and occupying 1097 ha, was established in 1990 and opened to the public in 1992. The FBG is established mainly for conservation, education, and research. This report aims to develop an action plan for FBG to implement these objectives. SWOT analysis was used to develop this action plan. To achieve the conservative objectives, FBG should enhance the communication with the local government and aboriginal people; monitoring populations of vulnerable plants; prioritize, collect, grow, and display the conserved plants; integrate the *in* and *ex situ* conservation actions. To achieve the education objectives, FBG should enhance the volunteer's organization; train the guides of travel agencies; promote the awareness of the importance of biological diversity to visitors, students, teachers, policy makers, and staffs through plant display, proper interpretation, and symposium or workshop; involve school teachers and experts to develop adequate education programs. The action plan should also be evaluated after a period of time to ensure their feasibility.

12.14.5. Innovation of site layout for *ex-situ* conservation in botanic gardens

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In Nanjing Botanical Garden there was a *Taxus chinensis* living collection of only 11 individuals, 5 male and 6 female, introduced in 1950's. After 45 and more years a new population of 461 plants originated from the collection occurred in the neighboring natural woods. Among all individuals 23 plants were more than 2 m high in 1999. Based on this fact and similar appearance of some other species authors suggested that small *ex-situ* conservation collection can developed into a new and large population if the integrate ecological conditions, environments with rich biodiversity in both physical and biological aspects, are provided. The current living collections in botanic gardens are basically or totally under cultivated conditions and they are substantially *ex-situ* cultivation but not *ex-situ* conservation because of lacking of appropriate ecological conditions. So that the layout of *ex-situ* conservation site should be changed and a site mixed with cultivated land and wild field is needed. It could bring about a revolutionary reform in botanic gardens.

12.14.6. The botanical gardens in Kew and Vienna and their role in scientific biodiversity research to provide tools for conservational decisions on Meliaceae (mahogany family)

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Pantropical Meliaceae (order Sapindales) have overwhelming economic and scientific-medicinal importance in both Europe and the USA, and one quarter of their species are listed as "threatened" in the IUCN 2003 Red List and are key target taxa in campaigns of international conservation groups. Taxonomic boundaries at and above species level in the family are, however, still largely unclear. Collections and expertise at the botanical gardens in Kew and Vienna provide valuable background for a project on Meliaceae and their links to conservation. We present the objectives and first results of the two-year (2004-2005) EU project "Threatened Biodiversity in Meliaceae: Urgent Need for a New Taxonomic Framework for Conservation" currently underway at RBGK. The project focuses on *Aglaia* (Southeast Asia, Australia and Pacific islands) and *Cedrela* (Central and South America). Objectives include contributing to the clarification of taxonomic delimitation at species level, creating the basis for setting up IUCN Red List assessments for target species, and evaluating hypotheses about the timing and causes of speciation.

12.14.7. International Plant Exchange Network (IPEN): A model for plant material transfer among Botanic Gardens

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Since the Convention on Biodiversity (CBD) asserted the sovereign rights of the countries over their genetic resources in 1992, Botanic Gardens that naturally depend on plant material transfer among each other face a completely new legal situation. The International Plant Exchange Network (IPEN), established by Botanic Gardens in order to comply with the CBD, covers only plant material transfer between Botanic Gardens and for non-commercial purposes. Botanic Gardens that want to join the network must adopt the IPEN Code of Conduct and use its common documents for plant material transfer. The benefit of participation in IPEN lies in the harmonized and simplified plant material transfer among members, without the need for individual material transfer agreements. Moreover the IPEN numbering system guarantees traceability and transparency: all plant material entering the network is given a unique and individual IPEN-number, containing the ISO-code of the country of origin and the acronym of the Botanic Garden introducing that plant material.

This Botanic Garden is obliged to keep record of all information regarding the origin of this individual accession.

12.15.1. Root system and water supply of plants in extremely arid conditions

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The plants of extremely arid regions have high absorb water properties of roots and water regulation by abilities. The roots of many hyperxerophytes has water reserve formation - root tubercle (*Scorzonera pusilla* Pall.), thickening (species of a *Calligonum*), sometimes such as a root crop (*Ferula caspica* M.B.), develop plentiful quantity epemeral roots in the damp period, have abilities to absorb inter condensation moisture. Some kinds develop root system reaching up to a level of earth waters (*Alhagi pseudoalhagi* (M.B.)Desv.or up to the zone of their capillary raising (*Ceratoides latens* J.F.Gmel.), even overcoming dry horizons of soil (*Kochia prostrata* L.Schrad). Species of plants, which roots do not reach these depths, have no a constant source of water supply during the whole period of the development, experience parched period and running in a status anabiosis or semianabiosis (species of a *Artemisia* from section *Seriphidium*).

12.15.2. Root growth and environment

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Field investigations on about 1.100 taxa showing morphology of the whole plant and on 900 showing anatomy of the root were done mostly in temperate zones of Europe including alpine regions, but also in subtropical and in desert areas outside Europe. Based on this the mechanism of geotropic growth was investigated by experiments in laboratory. The results are: The movement of water vapour by gravity inside and outside of plants is of great importance for their direction growth. Duhamel de Monceau explained this in 1758. The upward growth is connected with water supply for the elongation growth from inside, the downward from outside. Therefore the shoot covered with cuticula grows mostly upwards, the root without downwards. The root grows downwards, even in case of environmental resistance by higher and changing water supply, horizontally by less or constant water supply, and upwards by its lack. The root body is protected by root cap against too early water uptake. Due to environmental impact the root cap becomes often asymmetric. Consequently the uptake and loss of water including soluble substances within the root body are asymmetric too.

12.15.3. Structure of root endodermis within relationship between genetic and environment

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The endodermis is the most important part of the root cortex. The whole root cortex might be designated as a root buffer zone, especially under stress conditions. The endodermis, and to some extent the exodermis, represent apoplastic barriers. This function is a consequence of cell wall modifications in the first and the second developmental state. Improved technique for visualization of endodermis was developed. The development of endo- and exodermis influences the ion uptake processes of the root. The relationship between the accumulation and translocation of, and sensitivity to, cadmium and endodermal anatomy exists in willow clones. Moreover, the proportion of root apoplastic barriers, exo- and endodermis to other root tissues is increased in clones with higher tolerance to Cd ions. Endodermis at the third state protects the stele and vascular tissues in older root parts, and its function is already mostly mechanical. The extensive impregnation of endodermal cell walls by silicon takes place in some species during this state. Endodermal silicification has an impact on cell wall extensibility and the role in drought tolerance in some species is also shown.

12.15.4. Globalization of root demographics and function

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The functional dynamics of root growth and associated microbial contributions to nutrient and water uptake require additional

comparisons of root demographics and their life spans within soil profiles across geopolitical boundaries. Root contributions of carbon into the soil matrix enhance soil aggregate strength and improve additional root growth. Combinations of these feed-back associations with functional root dynamics across multiple long term ecosystems would provide valuable belowground information when cataloged by multinational scientific teams who compare specific species across soil and climatic landscapes. Comparative root functions require similar sampling and analytical protocols before libraries of spatial variabilities across numerous ecosystems can be developed. Soil models and root image processing are poised to initiate evaluations of functional root dynamics across many geopolitical boundaries. This new global root knowledge would provide essential information needed to increase the production and sustainability of food and fiber and to quantify root contributions to terrestrial carbon sequestration on planet Earth.

12.15.5. Responses of roots to soil water: Scaling from individual roots to global rooting depths

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Recent advances in research on gravitropic and hydrotropic root growth have allowed plant scientists to predict rates and directions of individual root growth and the spatial distribution of root systems, using data on the spatial and temporal distribution of soil water and using cost/benefit models of root growth. At a larger scale of populations and communities, optimization models that maximize plant water acquisition in the presence of root competition have yielded promising results. Insights gained from this research at smaller scales now allow us to scale up and predict global patterns of vertical root distributions. A simple soil-water balance model was used to successfully predict vertical root distributions under a wide variety of global vegetation types. The prediction that vertical root distributions are related to the soil water balance was further tested by using global root data sets to test for statistical relationships between rooting depths and seasonal patterns and long-term means of precipitation and evapotranspiration, as well as soil texture. The resulting empirical models were used to create global maps of predicted rooting depths.

12.15.6. The carbon cost of root function in boreal forests

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Studies on root biomass, growth and turnover are notoriously more difficult than studies of aboveground growth processes. Data from various European forest stands suggest annual turnover rates of 2-4 times the average fine-root biomass (see below). The root function is extremely costly and is enhanced by a high carbohydrate supply. The aboveground capacity to supply carbohydrates by photosynthesis governs the long-term root and mycorrhizal growth rate. Roots can grow in any direction even upwards (Kutschera et al. 2004). Roots occupying favourable soil regions tend to grow at the expense of others. Carbohydrates are stored at high levels in the fine roots; starch levels of 25-30% are frequently found. Tree roots can persist in the soil and survive for several years even when photosynthesis is restricted. References Kutschera L, Haas, D, Sobotik M. and Lichtenegger E (2004). *Carinthia II*: 117-130 Persson H (2000). *Developm Plant Soil Sciences* 87: 337-346 Persson H, Ahlström K (2002). *For Eco Manage*168: 29-41 Stober C, Eckart GA, Persson H (2000) *Ecological Studies* 142: 99-121

12.15.7. Effects of grazing on root biomass and demography in a Alpine grassland

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An experiment was undertaken to determine the effects of grazing on root production, mortality and decomposition in a mountain grasslands, and to assess the patterns of biomass allocation to roots under different environmental conditions and different management options. In fact, the vast majority of literature data concentrate on short-term studies and indicate that clipping consistently retards root growth; yet, field studies are few and contradictory, and tend to indicate that grazing has little effect on grassland belowground production. The experiment was

conducted throughout a 1-year-period in a high mountain cow farm ("malga") in Trentino (Northern Italy), at a height of 1700 m a.s.l using minirhizotrons installed in soil. Fine root production and mortality varied seasonally, with greatest production in spring and greatest mortality in winter. Root biomass was reduced sharply with depth and, in the ungrazed area, it was less abundant at the 5-10cm depth, and more abundant at 10-20cm, in comparison to the grazed area.

12.15.8. Development of roots of juvenile European Beech and Norway Spruce individuals under ozone stress in the crown and *Phytophthora citricola* infestation in the soil

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Root development is affected by air pollutants like O₃ and plant pathogen infections. Because plants respond to O₃ in a similar way as they respond to a pathogen attack, we hypothesised that juvenile beech and spruce under enhanced O₃ concentration are less susceptible to the root pathogen *Phytophthora citricola*. A two-year phytotron experiment was conducted with 32 planting containers, each hosting 10 beech and 10 spruce saplings. A natural climate was reproduced with ambient and twice-ambient O₃ regimes in combination with an inoculation treatment with *P. citricola*. Quantitative PCR was performed to verify infection. Root length, fine and coarse root biomass, tips per root length, root to shoot and fine to coarse roots relations were determined in beech and spruce to analyse effects by O₃ as well as the pathogen. Beech root development was reduced under enhanced O₃ or under *P. citricola* infection, but effects were not additive. Spruce root development was only reduced by combined stresses. We conclude that susceptibility to *P. citricola* under enhanced O₃ conditions is increased in spruce but decreased in beech.

12.16.1. The virtual information world: challenges for libraries

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Even while resources and access are expanding, librarians and information specialists face increasing challenges. The speaker will outline various types of electronic resources such as databases, electronic publications, scanned text and digitized herbarium specimens. Three main areas of challenges to libraries will be discussed: identifying and collecting electronic resources, providing access to the electronic resources for users, and preserving or archiving these resources. How can librarians find out what databases have been created or which texts have been scanned and converted into digital format? How can or should libraries provide access to these resources for their users? How do libraries ensure that once an electronic resource has been created users will always have access to it? What are some of the financial demands of handling electronic resources, and how can libraries manage these demands and maintain other library functions?

12.16.2. The African Plants Initiative: digitising and data-sharing through Aluka

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The African Plants Initiative (API) is a new network of institutions holding important collections of herbarium specimens of African plants and related materials. API partners commit to database and produce high resolution images of all their type specimens pertaining to African plants in the first instance and selected additional material of particular interest, including species of economic importance and regional or local endemics as well as non-specimen resources such as illustrations, texts and archival materials. The network has grown rapidly since its inception in mid-2003 and now includes 24 partner institutions in 15 countries in Africa, Europe and the US. By January 2005, more than 44,000 African specimens had been digitised by API partners, and it is envisaged that the majority of the estimated 300,000 African types in herbaria worldwide will have been digitised by mid-2006. The materials will be made available collectively by Aluka, a new, not-

for-profit initiative aiming to increase access to scholarly resources from the developing world, beginning in Africa, based on the successful model developed by J-STOR (<http://www.jstor.org/>).

12.16.3. Visual plants - a tool for local and web-based access to information of single images, specimen and species of plants

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Huge progress in biodiversity information systems on the web is evident and a very helpful source of information about specimen and species distribution. But access to the internet is still restricted to members of so-called developing countries. Visual Plants tries to fill this gap being an on-site tool for information retrieval and at the same time a web-based system. Information stored is hierarchically organized showing information for each single image, the specimen and the species. Additionally basic information about families is provided.

Visual Plants (visualplants.de) can be used as a tool for the visual determination of plants using basic (and mostly vegetative) characteristics of the specimen which is photographed in the field, scanned as fresh material or digitized as a herbarium specimen. It is already used in two research frameworks like the DFG funded research unit 402 "Functionality in a Tropical Mountain Rainforest: Diversity, Dynamic Processes and Utilization Potentials under Ecosystem Perspectives" (www.bergregenwald.de) and the BMBF funded framework BIOTA-East (www.biota-africa.org/1024/frames/biota-africa.htm).

12.16.4. The Solanum PBI project: taxonomy in the electronic information age

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We have begun a multi-institutional, multidisciplinary project to complete a species-level treatment of the genus *Solanum* (Solanaceae) by the year 2008. *Solanum*, with approximately 1500 species, is one of the largest and most economically important plant genera (it includes tomato, potato, and eggplant, among others). Our project, funded by the NSF PBI (Planetary Biodiversity Inventory) program, provides species descriptions, interactive keys, a nomenclature index, a photo and illustration archive with images of type specimens, an illustrated glossary, and a searchable database of herbarium specimens for all taxa in the genus. This information is disseminated electronically on our *Solanaceae* Source website. This project will create a globally accessible information source that will link libraries, collections, and electronic resources containing *Solanum* data. Such virtual resources are the way of the future in taxonomic research, in which far-flung and historical sources of information are synthesized. We expect this project to provide a model for other large scale taxonomic endeavors and for the electronic distribution of biodiversity data.

12.16.5. The Biologia Centrali-Americana Centennial: a vision for digital access to taxonomic information

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A major problem for access to biodiversity information is the many forms and places where it is stored. While data standards for storage and exchange of taxonomic names and specimen data are relatively stable, literature is still generally not accessible electronically or, if it is, the format is not one that enables interoperability with other data types. Research and products of a wide variety are hindered by the lack of easy access to taxonomic literature. The BCAC project aims to create a model to resolve these issues. The project will make a major taxonomic resource for Mesoamerica, the 58 biological volumes of *Biologia Centrali-Americana*, available in JPEG and PDF formats on the web and as a fully searchable resource. The model will be appropriate for all taxonomic literature and will enable interoperability with specimen databases, taxonomic authority files, and other datasets made

available on the web. In addition to integrating literature and other data sources, the method opens possibilities of generating further web products including checklists from multiple publications available in this form and a way of speeding production of taxonomic products.

12.16.6. PRISMA: Web integration of heterogeneous taxonomic information sources

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We will describe in this paper a tool developed to handle data for heterogeneous sources. At present, there are number information sources which although having related contents are dealt with independently: on one hand, the GBIF initiative, which aims to provide access to data from a vast number of [sources/data providers] under a common format, has manage side-by-side data from different types of collections, observations, surveys, etc. On the other hand, organizations such as Species 2000 intends to provide the nomenclatural backbone to enable the users with road map to all those names those appear in the primary data sources. The integration of names, concepts and specimens / observations is one of the biggest challenges of the management of biodiversity data. In this contribution we tackle the problem using (multi-agent system, ontology, probabilistic approaches, etc) to provide to the user a way to obtain meaningful responses from distributed biodiversity information systems.

12.16.7. Creating a digital information environment for USDA plant germplasm collections

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A collaborative project between librarians and botanists in USDA to integrate or link USDA databases containing information on USDA germplasm accessions of agronomic and horticultural plants is underway. Databases to be integrated are the Germplasm Resources Information Network (GRIN) and AGRICOLA of the National Agricultural Library (NAL), with additional USDA resources also involved. GRIN contains essential data on nearly 500,000 accessions and over 40,000 species, including passport, evaluation, descriptive, and taxonomic data, and useful data on agricultural plants in general. AGRICOLA is a database of ca. 4 million agricultural citations created by NAL and its cooperators describing information encompassing all aspects of agriculture and allied disciplines. Two elements controlled in both datasets that form the basis for this integration are scientific names and plant introduction (PI) numbers. This cross-linkage provides benefits to both databases and their users, including an increased user base, tracking of germplasm utilization, direct access to needed bibliographic or taxonomic information, and improved taxonomic standardization.

12.17.1. A landscape approach to the study of gene flow in plant populations

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Gene flow is a major determinant of plant genetic structure, and genetic structure analyses of plants at varying scales yield particularly useful insights concerning contemporary and historical gene flow. We will present a case study of wind-pollinated California valley oak, *Quercus lobata*, to illustrate several points about how to add a landscape perspective to population genetics. (1) We will examine contemporary pollen flow in Valley oak, using the TwoGener pollen pool structure method, and will test the assumption of isotropic pollen flow (no directionality), using spatial autocorrelation analysis. (2) We will deploy a seed pool structure analysis to identify the scale of contemporary seed movement, illustrating with seed transport by acorn woodpeckers. (3) We will use spatial autocorrelation analysis of adult Valley oak genotypes to estimate the scale of the historical neighborhood. (4) We will explain the spatial distribution of chloroplast microsatellite haplotypes in California valley oak with respect to historical

patterns of colonization and seed movement. These studies illustrate the insight we gain by examining gene flow in real landscape.

12.17.2. landscape genetics on different scales of space and time

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A new genetic variant initially exists in only one place at one time. DNA replication allows this variant to travel from one generation to the next and to spread through space. The strategies that work best in revealing how genes spread through space and time depend upon the spatial/temporal scale of the study. At small spatial scales, high levels of pre-existing genetic variation, such as those provided by microsatellites, are needed because the mutational process is not a major factor on a short time scale. Insight into the movement of genes through the landscape can be achieved by coupling microsatellite surveys with traditional population ecology studies. As the geographical and temporal scale of the study goes up, the mutational process becomes important, and phylogeographic approaches become increasingly informative. At intermediate spatial and temporal scales, both phylogeographic and microsatellite approaches are informative and complement each other. At the largest temporal scales, phylogeographic and coalescent approaches become the primary tools for analysis.

12.17.3. Genetic diversity and population structure of *Geum urbanum* L. (Rosaceae) in fragmented agricultural landscapes

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As a consequence of natural distribution of suitable habitats and anthropogenic fragmentation processes, many species occur in spatially structured populations. In the agricultural landscapes of Central Europe arable intensification resulted in major landscape transformations. Decreasing area of habitats and increasing isolation have severe effects on the viability of populations inhabiting the agro ecosystem. Avoidance of the unsuitable matrix and altered dispersal distances between habitat fragments may lead to increased inbreeding, thus to reduced genetic diversity. We studied the population structure of *Geum urbanum* L. as influenced by land use intensification and landscape fragmentation by means of microsatellites. We compare genetic population structure with GIS generated landscape metrics. The survey was conducted in three differently structured landscapes of Germany. Despite being mainly self-pollinated, *G. urbanum* populations were genetically diverse, irrespective of population size. Populations where highly differentiated. Single events of long dispersal can be traced by multilocus genotypes found in different populations.

12.17.4. SSRs in fragmented *Primula vulgaris* populations indicate the importance of a network at landscape level on fitness

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Primula vulgaris is a self-incompatible, perennial herb with two floral morphs: pins and thrums. In Flanders, (northern Belgium) the species is rare and declining. It is restricted to a network of fragmented populations in areas of intensive agriculture. Habitat fragmentation resulting from human disturbance reduces the size of populations and increases their isolation. The possible consequences are increased random genetic drift and inbreeding, and reduced gene flow between populations. This can lead to genetic erosion and increased genetic divergence between populations. We studied both adults and first year seedlings of *Primula vulgaris* populations for microsatellite loci at the landscape level. The genetic differentiation between adults and seedlings, as well as the significant effects of population characteristics and of the surrounding network of populations on the genetic diversity and the fitness will be discussed.

12.17.5. Why are some plant species restricted to river corridors?

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The restricted distribution of some plant species to river corridors (RC) has often been attributed to dispersal by water. Here, we present results showing that also soil and flooding conditions contribute to this distribution pattern. In a common garden experiment, we compared the performance of 18 species that are restricted to RC and 26 species that are not restricted to RC in NE Germany. Plants were grown in one non-RC (garden soil) and two RC environments (RC soil, flooding). The performance of all species was best in the non-RC environment. However, the performance of species restricted to RC was lower than the performance of the other species. This applied to both soil environments but the difference was less pronounced on the RC soil. Moreover, the species restricted to RC had a higher reproductive performance than the other species when they had been flooded. Our study suggests that one of the reasons that some species are restricted to RC is their competitive inferiority in non-RC environments but that in RC these species can compete successfully due to their better tolerance of RC-specific conditions.

12.17.6. Landscape genetics of *Stenocereus stellatus* (Cactaceae) in an edaphic crossequence of Tehuacan-Cuicatlan Valley, Mexico

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Genetic structure of *Stenocereus stellatus*, a columnar cacti with both sexual and asexual reproduction, in an edaphic crossequence of Tehuacan-Cuicatlan Valley was analyzed. We tested whether, due to humidity gradient in the four soils: 1) the subpopulations were genetically differentiated and 2) clonal recruitment had affected spatial distribution of genotypes in arid soils. The analysis was made using 75 RAPD markers. AMOVA showed a significant genetic divergence between the two subpopulations of humid soils and two arid ones ($F_{ST} = 0.16$, $P < 0.001$). Clonal recruitment determined the spatial distribution only in humid soils, according to isolation by distance model. Although the gene flow was high ($N_m = 4.29$), subpopulation divergence is probably due to seed recruitment through windows of opportunity as well as high recruitment of ramets in humid soils. Probably seed or ramets recruitments are not allowed in arid soils, long time ago. If the patterns of recruitment observed in humid soils occurred in the arid ones during the past, soils development could play an important role in the genetic structure and spatial distribution of genotypes of *S. stellatus*

12.17.7. Linking intra-specific diversity and habitat suitability - a case study using *Pinus cembra* L.

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Predictive habitat modeling is a means to explore the potential suitability of a landscape for a target species. By this, statistical models are formulated that allow expressing the suitability of any location in a landscape as a function of spatially explicit environmental predictors. We explore the potential of using habitat suitability of *Pinus cembra*, simulated as a function of proximal bioclimatic predictors, for explaining genetic diversity. We sampled 48 individuals at 20 locations throughout the Swiss Alps and assessed the number of chloroplast haplotypes per site, as well as the haplotypic diversity. We first analyzed the power of bioclimatic predictors to explain haplotypic diversity, and then we analyzed how much the predictive power changed when adding habitat suitability of *P. cembra* for explaining its local haplotypic diversity. We used ordinary least squares regression as well as generalized linear models. The statistical powers found may help to refine hypotheses of genetic diversity pattern as influenced by population processes and its effects on pollen and seed gene flow.

13.1.1. Molecular evolution of flower color determining genes: a model for the study of adaptive evolution

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The methodological difficulties associated with linking single gene change to adaptive phenotypic change are notorious, yet this is a central goal of evolutionary genetics. Flower color is a phenotype that simplifies many of these complexities. Well-established biochemical pathways and extensive molecular characterization of pathway genes illuminate some of the causal connections between

genes and phenotypes. Flower color is also a trait that has a direct affect on reproductive fitness and is therefore likely to be subject to strong selection. A plant genus that exhibits a rich array of floral color adaptations associated with speciation is the morning glory genus (*Ipomoea*). We present a comparative analysis of gene expression for flower color determining genes and we consider patterns of molecular evolution for pathway genes. Shifts in flower color phenotype among closely related species are frequently associated with changes in the regulation of gene expression rather than mutations in structural genes. We also show that structural genes in the pathway exhibit complex shifts in rates of molecular evolution throughout the evolution of the genus *Ipomoea*.

13.1.2. Population genomic studies in *Arabidopsis* and rice

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Population genomic analyses allow us to survey variation within genomes for evidence of selection, as well as to examine adaptation in a genomic context. High-throughput acquisition of sequence data, as well as the sequencing of entire plant genomes, has made population genomic approaches possible. We have been attempting to characterize the genetic basis of adaptation in *Arabidopsis thaliana* and *Oryza sativa*. Both of these selfing plant species have served as model genetic systems, and the availability of genomic resources in these species allow us to examine adaptation in a genomic context. This talk will discuss two studies. First, we will discuss a dissection of high-diversity genomic regions in *A. thaliana*, which may contain balanced polymorphisms. Second, we will examine levels and patterns of polymorphisms in the rice genome. Both these studies will illustrate approaches in identifying genomic regions that may contain genes underlying adaptive traits in plant species.

13.1.3. Gene expression variation in natural populations of *Arabidopsis*

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The relative importance of changes in coding versus regulatory sequences in adaptation and evolution is still unclear. Partly for technical reasons, most experimental work has focused on changes in protein coding sequences, for which rigorous model-based testing has been developed. Still, changes in gene expression are likely to constitute an important component of phenotypic evolution. A challenge for future research is to characterise variation in gene expression within and between species, and the underlying variation in regulatory sequences. We have initiated microarray studies of gene expression variation within and between plant species in the Brassicaceae family, with the aim to quantify gene expression in natural populations, and to assess to what extent this variation is important for adaptation. We will present data on global transcriptome variation between populations of *A. thaliana* and *A. lyrata*, as well as between the two species. We are also searching for gene expression differences that are associated with variation in apparently adaptive phenotypic variation (i.e., variation in flowering time, cold acclimation and trichome density).

13.1.4. Sequence diversity in maize (*Zea mays* L.): transposable elements, regulatory variation and heterosis

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The extensive lack of collinearity among allelic maize genomic segments has recently been described. Two types of phenomena are responsible for this observation: presence/absence of LTR-retrotransposons and presence/absence of genic fragments. Retrotransposons that are not shared between inbred lines appear to have inserted into the maize genome significantly more recently than those that are shared, revealing an active movement of high copy number elements in very recent evolutionary times. The genic fragment polymorphisms are also due to recent insertions of non autonomous elements of the *helitron* class. It has often been postulated that transposable elements are still actively reshaping genomes: the maize genome is in constant flux in that

transposable elements continue changing both the genic and non-genic fraction of the genome, profoundly affecting genetic diversity. In addition to the non colinearity we have detected extensive *cis*-regulatory variation in maize genes. We will discuss the implications of the allelic non collinearities for regulatory variation, heterosis and evolution of novel gene functions.

13.1.5. Genome-wide analyses of the evolutionary impact of transposable elements

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Once dismissed as purely selfish, the inherent ability of transposable elements to move and replicate enables them to participate in a variety of phenomena, from genome plasticity to new gene formation. As the single largest constituent of many genomes, investigations of transposons must often be conducted at genomic scales. We have undertaken large scale analyses of the genomes of several completely sequenced organisms, including rice and *Arabidopsis*. We review current understanding of transposons' role in evolution and describe challenges and rewards of genome-wide transposon studies. Challenges include devising methods to deal with the repetitive, diverse, and degenerate characteristics of transposons. Rewards include characterizing important evolutionary phenomena such as transposon-mediated gene duplication ("transduplication"), selfish gene "domestication", host gene capture, and phenotypic impacts of new insertions.

13.1.6. Opposing evolutionary dynamics on linked genes in a disease resistance pathway in wild tomato

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Understanding the evolution of genetic pathways requires investigating how selection is acting on each individual component of the pathway. I have investigated the selective forces which have shaped the evolution of two genes required for resistance to a bacterial pathogen of tomato, *Pseudomonas syringae* pv. *tomato* in a natural population of the self-incompatible tomato species, *Lycopersicon peruvianum*. The two genes are *Pto*, which has been identified as the receptor for the pathogen ligand, and *Prf*, which is closely linked and required for activating the resistance response once the pathogen has been detected. Of fifteen genes surveyed, *Pto* had the highest amino acid polymorphism, consistent with either relaxed selective constraint or the maintenance of amino acid variation by some form of balancing selection. In contrast, the low level of amino acid polymorphism at *Prf* is consistent with purifying selection operating at this locus. These results represent a first step towards understanding how potentially opposing selective pressures are reconciled at the molecular evolutionary level.

13.1.7. Molecular evolution of the gene NORK in the model legume *Medicago truncatula*: positive darwinian selection in a mutualism.

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Understanding the selective constraints of partner specificity in mutually beneficial symbiosis is a significant, yet largely unexplored, prospect of evolutionary genomics. These selective constraints can be explored through the study of nucleotide polymorphism at loci controlling specificity. The membrane-anchored receptor NORK (nodulation receptor kinase) of the model legume *Medicago truncatula* controls early steps of root infection by two symbiotic microorganisms: nitrogen-fixing bacteria and endomycorrhizal fungi. We analyzed the diversity of the gene NORK in a set of inbred lines sampled from natural populations. Tajima's D and Fay and Wu's H revealed no departures from the neutral model. We analyzed divergence using sequences from the closely related species *M. coerulea* and found an excess of non-synonymous changes contributing to this divergence. Maximum-likelihood analysis of a molecular phylogeny of legume species indicated that some sites, likely located in the receptor domain of the protein, evolved under positive selection. These results as well

as patterns of linkage disequilibrium a region of ~120 kb flanking NORK will be discussed.

13.2.1. Epidermal patterning in *Arabidopsis*: Trichomes as a model

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The spacing of trichomes in the leaf epidermis of *Arabidopsis thaliana* is a well-studied epidermal patterning system. The spacing of trichomes is established by a conserved gene cassette consisting of a myb R2R3 factor, a basic helix loop helix factor and a WD40 protein. These proteins act together to promote trichome development and are counteracted by single-repeat myb factors that are believed to move between cells to mediate cell-cell interactions. The logic of the system is assessed by several approaches: First, the genetic interactions and the expression of the involved genes are analysed. Second, the promoters of positive and negative factors are compared to determine qualitative and quantitative differences. Third, the cell-autonomy is analysed using the Cre-Lox system. Fourth, the relevance of the cell-autonomy or non-autonomy is tested by manipulating the autonomy of the individual proteins.

13.2.2. Integrating molecular systematics, morphology and anatomy towards understanding evolution of Kranz anatomy in the genus *Flaveria* (Asteraceae)

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For most C₄ plants, Kranz anatomy represents a critical set of structural features that facilitates C₄ photosynthesis. Many studies describe Kranz anatomy; however, little is known how these anatomical characteristics have evolved in relation to each other and to C₄ biochemistry. One of the best models for studying C₄ evolution is the small New World genus *Flaveria* (Asteraceae) which includes C₃ and C₄ species, in addition to species classified as biochemically intermediate (C₃-C₄ or C₄-like). To date, anatomical studies of *Flaveria* are limited and a complete phylogeny does not exist. We use molecular (cptrn L-F, nrITS and nrETS DNA sequences) and morphological data to reconstruct the phylogeny of *Flaveria*. We then mapped Kranz anatomy and vein pattern data from a comparative leaf anatomy study of *Flaveria* species (C₃, C₄, C₃-C₄, C₄-like) onto the phylogeny. The results indicate that C₄ (or C₄-like) photosynthesis has evolved independently within two clades of this genus. Moreover, these data suggest that evolution of Kranz anatomy traits proceeded in a step-wise manner within each clade.

13.2.3. Gilding the lily: the evolution and development of specialised petal cell shapes

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Specialised epidermal cells with particular forms interact to enhance the attractiveness of flowers to their pollinators. We have been working on the development of several specialised cell types in the Antirrhinum petal, which are linked through the activity of a small group of MYB transcription factors. We are now extending this work to investigate the evolution and development of specialised petal cell shapes in other species, including basal Angiosperm groups and species with a range of pollination systems, including buzz pollination. This work also involves assays of pollinator behaviour in response to different petal cell types. We have also recently begun work on the roles of specialised petal cells in producing the fly-mimicking "beetle spots" of *Gorteria diffusa*. These spots are composed of localised anthocyanin deposition and specialised papillate cell shapes.

13.2.4. Morphology of pumpkin leaf trichomes and their ecophysiological roles

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Plant trichomes are involved in a high number of different functions like secretion processes, control of water loss, protection from heat, etc; especially glandular trichomes show high functional amplitudes. Leaves of Styrian oil pumpkin (*Cucurbita pepo* L. var. *styriaca* Greb.) develop three glandular trichome types (I, II, IV) and one non-glandular (III) trichome.

The structural differences of these trichomes were studied by means of light and electron microscopy. Histochemical staining reactions and cadmium experiments were carried out in order to get more detailed information about the major functions and secretion processes of the trichomes.

The light microscopical data showed differences in the ontogenesis and histochemistry of the trichomes, as well as in the secretion processes. Ultrastructural analyses gave continuative information and indicated changes in the vacuolar deposition and altered plastids depending on the cell type and secretion stage. Treatment with cadmium did not induce ultrastructural changes and no indication for the involvement of these trichomes in detoxification processes could be found.

13.2.5. Regulation of egg cell identity in the female gametophyte of *Arabidopsis*

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In plants the egg cell is formed by a separate haploid generation, the female gametophyte. The female gametophyte of *Arabidopsis* develops from a single haploid spore. This spore divides mitotically to give rise to seven cells, one of which is the egg cell. We study the mechanisms that underlie the specification and regulation of egg cell identity. We made use of an egg cell specific marker line and screened for mutants with a deregulated expression of the egg cell marker.

The *lachesis* mutant expresses the egg cell marker ectopically in cells neighbouring the egg cell. Morphological, molecular and functional data indicate that the respective gene is necessary for restricting egg cell identity to the egg cell only. In addition, our data suggests that cell-cell communication is vital for the specification of different cell types in the female gametophyte. A phenotypic characterisation of the mutant and the potential function of the encoded protein will be discussed.

13.2.6. The role of spatial constraint on the ontogenesis of stomata: a case in Velloziaceae

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The stomatal types have long been used as characters in systematics. However, identical stomata can have distinct origin, and a similar ontogenetical pattern can form different stomata. Stomatal ontogenesis of four Velloziaceae species, *Nanuzia plicata* and *Vellozia epidendroides* (tetracytic), and of *V. glauca* and *Barbacenia riparia* (paracytic), were investigated. The stomata follow the perigenic development. The subsidiary cells arise from oblique divisions of neighbor cells of the GMC, which are elongated parallel to the longer axis of the stoma. Polar cells show wide variation, following the shape and size of the epidermal cells in the vicinity, and so cannot be called subsidiary cells. High concentration of GMCs forces the development of short polar cells, leading to an apparently tetracytic stomata; low concentrations allow the elongation of the polar cells, thus leading to true paracytic stomata. The space available between the GMCs can interfere in the final configuration of the mature stomata. Therefore, the four species were considered braquiparacytic and the former stomatal classification in Velloziaceae must be questioned.

13.3.1. Processes of evolution in the flora of southern South America

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The flora of southern South America has developed from indigenous elements, taxa that arrived from Australasia, Africa, or North America, as well as neotropical elements that moved into the high latitudes. The timing of the establishment of non-native taxa depended on the development of suitable habitats. Likewise radiations of non-native and indigenous taxa depended on the shifting patterns of geology and climate that determined the temporal and spatial patterns of the habitats we now see in southern South America. The relative importance of vicariance and dispersal as the factor determining the influx of taxa depended on habitat and to some extent life history. Recent molecular phylogenetic studies have shown that many of the elements in the

southern flora are the result of radiations of native stocks but for herbaceous and shrubby taxa shared with Australasia, Africa, and North America, long-distance dispersal appears to have been the dominant mode of movement between areas.

13.3.2. Phylogenetic and biogeographic patterns in the endemic South American subtribe Myrciinae (Myrtaceae)

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Myrtaceae distribution reflects ancient land links between Australia and South America, with centres of diversity on both continents. It has been proposed that Myrtaceae progenitors arose in Gondwana previous to the separation of Africa, radiating in what is now Australia. Subsequent land bridges joining Australia, Antarctica and South America allowed the family to extend its distribution to the latter continent. Phylogenetic relationships of Myrciinae (the only subtribe of Myrtaceae endemic to South America) reconstructed using *psbA-trnH*, ETS and ITS sequence data support this theory. Indications are that Australian taxa are sister to South American taxa while monophyletic Myrciinae is nested within the South American clade. Short branch lengths at generic and subtribal levels in Myrciinae suggest a rapid and early radiation in this group. Some genera difficult to assign to subtribe on account of their anomalous morphology have disjunct distributions restricted to a few temperate localities (e.g., on either side of the Andes and the Juan Fernandez islands). It has been suggested that these taxa are unspecialised early branches from Myrtaceae ancestors.

13.3.3. Speciation of *Hypochaeris* (Asteraceae) in South America: a model for continental adaptive radiation

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The herb *Hypochaeris* (Asteraceae, Cichorieae) has diversified into c. 40 species in South America after dispersal from NW Africa in the Pliocene or Pleistocene. It has colonised all regions of the continent except the moist tropics, being found in subantarctic regions in Argentina and Chile northward into subtropical Brazil, and even further north into the high Andes as far as Venezuela. Species occur from sea level to more than 5,000 m in different habitats. Morphologically these species exhibit a wide variety of forms, ranging from large-leaved herbs 1 m tall to rosette-leaved species only a few centimeters high. Amplified fragment length polymorphism (AFLP) data help differentiate parallel lineages that have evolved rapidly in response to different ecological conditions. The pattern in *Hypochaeris* of South America, therefore, is of rapid speciation via adaptive radiation on a broad continental scale. The young age of the entire group (c. 1 million years) suggests that this radiation might also have been impacted by Pleistocene glacial cycles.

13.3.4. Nothofagus biogeography and the Gondwana break-up

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The break-up sequence of Gondwana played an important role for biotic distribution in the Southern Hemisphere, resulting in vicariance events. In vicariance biogeography, areagrams have been used in order to find and explain such events. Areagrams may also convey on biogeographic information to the extent that alternative and unsupported palaeogeographic hypotheses may be enforced as alternative area relationships. Extinctions and dispersals are biological realities, albeit often difficult to assess, that may unfortunately distort the biogeographic signal, leading to an incomplete description of past distribution, and subsequently flawed areagrams.

By reconciling phylogenies with hypotheses of area relationships and optimising fossil data on the reconstruction, hypotheses of vicariance, dispersals, and extinctions can be tested. Fossils then become indispensable tools as evidence of past distribution and shall not be underestimated. The only palaeogeographic hypothesis explaining *Nothofagus* known past and present distribution, including that in Antarctica, is the current accepted model of Gondwana break-up.

13.3.5. Genetic diversity and differentiation within and among Chilean populations of *Araucaria araucana* (Araucariaceae) based on AFLP markers and allozyme variability.

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Genetic diversity within and among Chilean *Araucaria araucana* populations was estimated using AFLP markers and allozyme variability. Both kind of data were congruent, indicating that *A. araucana*'s genetic variability is relatively high compared with other biologically similar species. Most of the total variation was nested within populations, but a considerable interpopulational variation was obtained. Intrapopulational variation was considerably higher in Coastal population. Populational differentiation was highest between the north Andean population and the remaining populations. The patterns of actual genetic variation could be influenced by historical factors such as tectonic and paleoclimatic changes that occurred in southern South America. Different areas in Coastal and Andes Mountain remained as refuge during the Pleistocene Glaciations. From these areas the glaciated surface was recolonized. We discuss about genetic variability from a conservation point of view for *Araucaria araucana* forest in Chile.

13.3.6. Biogeographic history of *Nothofagus*- the molecular story

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Southern Hemisphere biogeography is now dominated by many examples of transoceanic dispersal. Even within the genus *Nothofagus* (Southern Beeches), which has become iconic for ancient Gondwanan relationships that date back to the Cretaceous era, dispersal has been proposed for some species. This view is consistent with recent results from molecular clock analyses of Australian and New Zealand Southern Beech species. Nevertheless, different molecular clock divergence time estimates for South American and Australian *Nothofagus* lineages appear to be consistent with the timing of the break-up of Gondwana. This raises the possibility that at least some Southern Hemisphere relationships can be explained by vicariance. Thus our results indicate that the biogeographic history of Southern Beeches is more complex than envisaged under opposing polarised views expressed in the ongoing controversy over the relevance of dispersal and vicariance for explaining plant biodiversity.

13.3.7. Molecular phylogenetics and historical biogeography of *Oreobolus* (Cyperaceae)

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Nuclear ITS and plastid trnL intron and trnL-F intergenic spacer regions were sequenced for 13 species of *Oreobolus* (Cyperaceae) from throughout its distribution range (South America, Australasia, and Hawaii), plus the monotypic genus *Schoenoides*; *Costularia laxa* was used as outgroup. Phylogenetic trees were produced for the datasets separately, as well as combined. We estimated species divergence times by enforcing a molecular clock on the combined tree, using the appearance of *O. furcatus* in Hawaii, no earlier than 5.1 mya, as a calibration point. Our results support the monophyly of the South American species with the southern *O. obtusangulus* as sister to the rest. This South American clade is sister to the Australian *O. pumilio*, and the remaining Australasian and Hawaiian species form a basal grade. *Schoenoides oligocephalus* is embedded within *Oreobolus*. The topology of the combined analysis indicates a recent (5.5--6 mya) origin of the South American clade, followed by a northward biogeographical progression of species along the Andes.

13.4.1. Concepts matter: characters as units of evolutionary change

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If organisms would be completely integrated wholes, biology as an analytical science would not be possible. Perhaps even evolution by mutation and natural selection would be impossible. In this contribution I will be arguing that the existence of well defined, quasi-independent characters is as fundamental to biology as the existence of species. Hence, for biology understanding the genetic/developmental organization of characters, their evolutionary origin and mode of evolution are important research objectives. The research questions associated with this field, among others, are: 1) what are the genetic/developmental mechanisms that constitute morphological character as quasi-independent units of evolution? 2) how does one recognize the existence and the limits of characters? 3) why are characters stable in the face of adaptive evolutionary change? 4) what are the mechanisms that lead to novel characters (evolutionary novelties)? 5) what are the constraints on character evolution, and what are their causes? I will discuss examples to illustrate research approach to address these questions.

13.4.2. Complexes of characters provide important insights into the function and evolution of pleurocarpous mosses

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Whether characters evolve independently or in more or less stable complexes has implications for phylogenetic analyses and for our understanding of their biological functions. Pleurocarpous mosses have important complexes of correlated states related to the spore capsule, its peristome, and calyptra. These complexes may include more than 20 states. Spore dispersal apparently depends on a much more intricate set of many co-evolving morphological structures than any found gametophyte function. Some of these complexes of states can be explained by the phylogenetic history of the taxa having them. Such complexes, and those that evolved repeatedly as a more direct response to the habitat, are all correlated with specific habitats and are therefore likely to be functional under extant environmental conditions. Wind speed, air humidity, and substrate moisture for spore germination are likely to be important factors for explaining the function of complexes of capsule and calyptra structures.

13.4.3. Reconstructing common ancestors using mapping of qualitative and quantitative characters: a case study of Apiaceae tribe Scandiceae

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We have reconstructed general fruit appearance for the ancestors of Scandiceae subtribes Scandicinae, Torilidinae and Daucinae, as well as of their major subclades. We considered all qualitative and quantitative traits that unambiguously characterise general fruit morphology and anatomy, including the shape and size of the fruit and its appendages and internal structures. Quantitative traits were re-coded as ordered characters based on the analyses of their distributions. All characters were mapped on the tree inferred from rDNA ITS sequences and the character states for the ancestors of particular subtribes, major clades, and genera were inferred. These reconstructions were generally unambiguous for most clades recognised in Scandicinae and Torilidinae, but not for Daucinae. Based on these ancestral character sets drawings of fruits were prepared. Such a visualisation may provide heuristically valuable insight into the evolution of plant form and function, and be better than the standard analysis of character evolution. However, since it depends much on the accuracy of ancestral character reconstruction, its utility is somewhat limited.

13.4.4. A contribution to solving the problem of morphological character states

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A contribution to the problem of creating morphological character states can be made by applying insights from cognitive psychology to the character state creation task. Psychologists have identified two visual processing modes. Analytic visual processing involves attending to distinct, separate features of a trait (e.g., septa, locules, and ovules of an ovary). Holistic processing involves attending to global features such as overall shape (Gestalt), or to interdependent aspects such as the distance between features. Preliminary evidence suggests that holistic processors are able to create character states with higher inter-investigator agreement (higher reliability), and higher agreement with an independent assessment of phylogeny (greater validity), than analytic processors. Holistic visual processing can be stimulated by training. To construct character states, photographs of traits are sorted into hierarchical groups. The results are hypotheses of the relationships among taxa based on a specific set of traits (a specific character). These hypotheses can be coded for phylogenetic analysis by using matrix representation with parsimony.

13.4.5. A penalty of using anonymous dominant markers (AFLPs, ISSRs, and RAPDs) for phylogenetic inference

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AFLPs (and to a lesser extent ISSRs and RAPDs) are increasingly being used for phylogenetic inference among closely related species, using both distance and parsimony analyses. The justification that is generally given for using these characters is that nucleotide sequences lack sufficient variation among the sampled taxa. Presence/absence characters for each AFLP allele treat all absences as homologous to one another. This is analogous to coding four nucleotides at a given position as four separate characters. When only two alternative alleles for each locus occur in the study group, this character coding is not particularly problematic because any change between alleles simply gets weighted twice. The problem arises with three or more alleles. We conducted a simulation study to quantify how severe the negative effect of using presence/absence characters of individual bands is for phylogenetic inference relative to standard multistate characters. We examined alternative tree topologies, relative branch lengths, numbers of characters, rates of evolution, and numbers of alternative alleles, using both parsimony and Nei-and-Li distance analyses.

13.4.6. Homology and character states in morphological and molecular data

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Phylogenetic analysis inherently consists of two phases. First a data matrix is assembled, then a phylogenetic tree is inferred from that matrix. The first phase of phylogenetic analysis is the most important; but paradoxically, by far the largest effort in phylogenetic theory has been directed at the second phase. Our goal is to examine each of the logical elements in the assembly of the data matrix: the rows (what are OTUs?), the columns (what are characters?), and the individual entries (what are character states?). There is never a given set of OTUs or characters to begin a phylogenetic analysis with. Furthermore, there is a reciprocal relationship between OTUs and characters. OTUs need to be constructed of semaphorons using character evidence, and characters need to be discovered and evaluated, during each analysis. A good taxonomic character shows greater variation among OTUs than within -- each must be a system of at least two discrete transformational homologs, or character states. This variation must be heritable and independent of other characters. These principles of character analysis apply to all sources of data, whether morphological or molecular.

13.4.7. Geometric spaces and quantitative methods for the similarity test of homology and character state identity

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Assessment of morphological homology consists of comparability statements and conjectures of character states as potential taxic

and transformed homologues. Statistical analyses are used in the test of similarity of homology for character state identity. Measurements or coordinates of fixed or varying "morphs" constitute clouds of vector points respective to a multidimensional geometric space configured by multiple axes. Identification of the same phylogenetic state (taxic homology) or recognition of sufficiently different states (transformational homology) is a statistical question about structure of correlations and variance in such vector space. We use analyses of variance to test if there is a statistically significant association of the quantitative feature (a character state) with the a priori discrete groups (species as sampling units). Multiple comparison tests are used to discover which groups of means are significantly different from each other. We exemplify this approach with mosses, orchids, bromeliads, diatoms and bats. Conjectures of character states are still hypotheses of primary homology, which need to be tested for congruence.

13.5.1. Northern Hemisphere deciduous forest disjunctions, with special reference to Dipsacales

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In recent years there has been a significant increase in the number of phylogenies for disjunct taxa, which has improved our understanding of phytogeographic connections in the Northern Hemisphere. We will review phylogenetic work to date and examine how temperate deciduous forests of the Northern Hemisphere have assembled during the Tertiary. Our discussion will focus particularly on ancestral areas, examine the timing of divergences, general patterns and possible causes, and compare with selected animal clades. Multiple examples of several Northern Hemisphere disjunction patterns are present within the Dipsacales (Asteridae). These patterns will be discussed and special emphasis will be given to *Viburnum* (Adoxaceae) and *Lonicera* (Caprifoliaceae), case studies that will provide the opportunity to present and discuss a database we are developing to assemble and analyze our knowledge of Northern Hemisphere biogeography.

13.5.2. Northern Hemisphere tropical lineages, with special emphasis on Leguminosae and Cucurbitaceae

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Molecular phylogenetic studies involving dense sampling at the species level within the legume and Cucurbitaceae families (worldwide) are revealing a remarkable level of geographic and ecological phylogenetic structure. Many intracontinental and transcontinental crown clades appear of Neogene to Quaternary age, pointing to migration across Beringia or the North Atlantic. Similar patterns exist in other families that today have disjunct tropical ranges. These patterns plus paleobotanical data (esp. new fossils) and phylogenetic studies that include molecular dating, reveal a picture of the boreotropics (Wolfe 1975) that includes seasonally dry biota bordering the Tethyan seaway, from Southeast Asia west to the Gulf of Mississippi and through North America, as well as mixed forests of a taxon composition that we are only beginning to fathom. Our contribution will focus on components of the Laurasian thermophilic flora that are predicted by molecular phylogenies, but are not yet found in the fossil record.

13.5.3. Tertiary intercontinental disjunctions in the phytogeographic history of the Northern Hemisphere

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The fossil record reveals patterns of intercontinental disjunction among Tertiary woody seed plant genera that help to explain modern phytogeographic ranges in the Northern Hemisphere, for example: East Asia-western North America (Amersinia, Craigia, Davidia, Deviacer, Dipteronia, Florissantia, Trochodendron, Tetracentron); Europe-North America (Buzekia, Cedrelospermum, Crucifera, Eostangeria, Tetraclinis); Europe-Asia (Banisteriocalyx, Buxus, Podocarpium, Pteroceltis). Some formerly pan-north temperate taxa are now either disjunct between North America and East Asia, or endemic to one of these regions (Carya, Cercidiphyllum, Comptonia, Liriodendron, Metasequoia,

Sequoia). Some of the Eocene Euro-North American genera are now confined to East Asia (*Diplopanax*, *Platycarya*, *Mastixia*, *Tapiscia*) or to Asia plus Africa (*Alangium*, *Ensete*, *Pyrenacantha*, *Tinospora*). European Tertiary genera now confined to tropical America include *Anacardium*, *Ceratozamia*, *Tetrapterys*, and *Matudaea*. The timing and directionality of the exchanges with Africa and South America remain to be documented.

13.5.4. Colonisation dynamics and the tempo of tree evolution in the northern hemisphere

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Phylogenetic and phylogeographic studies point to heterogeneous rates of molecular evolution across lineages in all kingdoms. This DNA sequence rate heterogeneity is attracting more and more interest, and mechanistic models based on generation time, metabolism, population size, diversification rate... are being actively developed. Here, I explore the idea that mutation rates are inversely related with the geographic stability of the corresponding lineages, using case studies relying on interspecific and intraspecific phylogenies of forest trees and shrubs from the northern hemisphere. In particular, I ask if species or populations within species that have experienced long-term geographic stability (geographic relicts) typically have more ancestral characters than their more invasive counterparts. The results suggest that data on the tempo of molecular divergence could be better valorised in historical biogeographic studies.

13.5.5. Patterns of morphological differentiation among intercontinental disjunct plants in the Northern Hemisphere

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I examine patterns of morphological differentiation in several intercontinental disjunct genera: *Aralia*, *Carpinus*, *Corylus*, *Dendropanax*, *Kelloggia*, *Oplopanax*, *Panax*, *Phryma*, *Prunus*, *Rhus* and *Altingiaceae* in a phylogenetic framework. There is evidence for higher rate of morphological differentiation at the junction between the tropics and the temperate region (e.g., in *Aralia*, *Altingiaceae* and *Prunus*). *Aralia*, *Panax* and *Carpinus* have highly distinct morphological groups in North America, whereas their species in Asia are morphologically complex, with several species complexes difficult to differentiate. Molecular data in conjunction with fossil evidence suggest a young age of these species complexes. In the same groups, there is evidence for highly distinct lineages in Asia. This morphological pattern supports eastern Asia as an active speciation pump as well as a relict "museum." Several disjunct genera are morphologically highly distinct from their closest relatives, yet the disjunct species have differentiated little. Morphological similarity of the disjuncts may be attributed to either young age, or stasis, depending upon the lineages.

13.5.6. The ecological context of evolution, speciation, and extinction in Northern Hemisphere disjunct plant genera

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Disjunct genera in eastern Asia (EAS) and eastern North America (ENA) illustrate independent evolution in regions having similar climates differing in geography. We investigate conservatism of physiological traits by comparing temperature and precipitation extremes within the ranges of genera in each region, and relate ecological breadth to species richness. Herbaceous genera exhibit stronger correlations between EAS and ENA, compared to woody genera, in response to climate variables and in area of geographic distribution. Primarily temperate genera that extend their distributions into the tropics differ between EAS and ENA, suggesting idiosyncrasy in adaptation to new environments. Rate of diversification is uniformly higher in EAS, presumably due to the greater topographic and geographic complexity of the region. With additional phylogenetic and ecological work, these disjunct genera will become an important model system for investigating diversification and adaptation in plants.

13.5.7. Comparison of rates of speciation and molecular evolution in Eastern Asia and Eastern North America

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Although similar in climate and floristic composition, eastern Asia (EAS) has twice as many species as eastern North America (ENA) in genera occurring in both regions. To explore causes underlying the diversity anomaly, rates of net speciation and molecular evolution between EAS-ENA sister clades were compared. Results from analyses of ten angiosperm genera and ITS sequence data revealed a pattern of greater species diversity and higher ITS substitution rate in the EAS counterparts. The results imply greater net speciation and accelerated molecular evolution in EAS. Fossil evidence from the compared genera does not suggest greater species extinction in ENA, supporting the idea that accelerated speciation in EAS, promoted by its highly dissected topography, is an explanation for the greater species diversity in EAS. The close relationship between rates of ITS evolution and species richness further suggests a link between rates of species production and nucleotide substitution. This hypothesis is further tested in *Cornus* with molecular data from other genes.

13.6.1. Bryophyte phylogeny: pushing the molecular and morphological frontiers forward.

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Revolutionary new concepts of bryophyte relationships have emerged from molecular phylogenetic analyses conducted since the onset of the 21st century. For example, sequence data contradict the historical notion that isophylly in leafy liverworts is plesiomorphic and that simple thalloid liverworts are monophyletic. Also contrary to traditional views, is the concept that *Leiosporoceros* is genetically distinct from other hornworts, and that *Oedopodium* is sister to the peristomate mosses. Substantial increases in comparative ultrastructural and anatomical data likewise have provided new insights on interrelationships. Because of this recent deluge in evolutionary studies on bryophytes, it is an opportune time to examine contemporary morphological knowledge in light of novel molecular hypotheses. Accordingly, this presentation will reevaluate the evolution of structural and developmental design in bryophytes, especially within the monosporangiate sporophyte. Examination of bryophytes is essential to identify structural innovations that accompanied land invasion and to resolve the evolution of more complicated body plans in tracheophytes.

13.6.2. A comprehensive molecular phylogeny of liverworts (Marchantiophyta)

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Sequence data from three chloroplast genes (*rbcL*, *rps4* and *psbA*), one nuclear gene (the ribosomal LSU) and one mitochondrial gene (*nad5*) were assembled for ca. 180 species (120 genera) of liverworts, providing the most comprehensive molecular data matrix for the group to date. Phylogenetic analyses of these data provide support for the monophyly of the liverworts, and resolve critical nodes along the backbone of Marchantiophyta. The earliest diverging lineage comprises the controversial taxa *Haplomitrium* and *Treubiaceae*. A *Blasiaceae*/complex thalloid clade resolves as sister to all remaining liverworts. The leafy liverworts do not resolve as monophyletic: the separation of the *Aneuraceae*/*Metzgeriaceae* from all other simple thalloids, and their placement within the 'leafy' clade as sister to the enigmatic leafy genus *Pleurozia*, as suggested in earlier molecular phylogenies, is also supported by this far larger data set. Within both the complex thalloid and the 'leafy' clades, however, there is little support for lineages.

13.6.3. Progress and Challenges in Reconstructing Hornwort Phylogeny

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Despite recent interest in the hornworts, taxonomic boundaries at nearly every level remain controversial. Molecular sequence data has been obtained from a plastid (*rbcL*) and mitochondrial (*nad5*) gene for more than 30 hornwort species representing eight named generic segregates. Phylogenetic analyses of these sequences reveal a number of hierarchical relationships that are not reflected in current classifications. In particular these analyses suggest three divergent lineages of hornworts which include: 1) the monotypic genus *Leiosporoceros*, 2) members of *Anthoceros* and *Folioceros* and 3) all of the remaining hornwort genera. Both morphologically and genetically distinct from all other hornworts, *Leiosporoceros* generally is resolved as sister to the hornworts however divergent rates of RNA editing among lineages of hornworts in both chloroplast and mitochondrial sequences create challenges for phylogenetic reconstruction. The third group includes a number of surprising relationships including the presence of at least one previously unrecognized group consisting of members usually ascribed to the genus *Phaeoceros*.

13.6.4. Inversions and length mutations in chloroplast DNA and their phylogenetic utility

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Non-coding DNA and fast evolving coding regions often display impressive degrees of length variation. Although these regions are widely used in phylogenetic analyses, their mutational dynamics and resulting patterns of variability are still incompletely understood. Length variation is analysed in several datasets with dense sampling for bryophytes (trnT-F, psbT-H, atpB-rbcL) or basal angiosperms (trnT-F, petD, trnK/matK) and compared across land plants (trnT-F, psbT-H, trnK/matK). It appears that inversions are largely associated with hairpins. Depending on their structural positions, inversions are often highly homoplastic unlike other kinds of microstructural changes (e.g., simple sequence repeats). Utilizing secondary structures and minimum free energy models, slipped-strand mispairing as a potential mechanism that creates duplications is evaluated for different structural situations.

13.6.5. Infra-specific molecular phylogeography of bryophytes

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Recent advances in DNA technology, along with the development of coalescent theory, provide very powerful tools for the study of population and speciation processes in bryophytes. They provide opportunities for testing hypotheses regarding population history and demography as forces shaping observed patterns of distribution and abundance as well as of genetic variation. We review coalescent theory with a particular emphasis on the opportunities and challenges that bryophytes offer. Areas that we particular focus on include i) species concepts and their relationship to gene genealogies, ii) the influence of past climates on distribution patterns, iii) conservation genetics, and iv) inferences about mating systems and reproductive biology. We review data from studies of bryophytes (mosses, liverworts, hornworts) that include sampling from multiple populations within species, and utilize molecular data based on nucleotide sequencing and various approaches to DNA fingerprinting.

13.6.6. The Moss Diversity Project: Haplolepideae (Dicranidae)

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As part of the Moss Diversity Project we focus in this paper on the relationships and diversity of Haplolepideae (Dicranidae). The Dicranidae are a major lineage of arthrodontous mosses with a unique peristome architecture, consisting of (when typical) a single ring of peristome teeth, with positional homology to the endosome of diplolepidous mosses. Phylogenetically the Dicranidae are most

closely related to the diplolepidous-alternate peristomate mosses (Bryidae), and are typical classified into 238 genera containing approximately 4,000 species. We analysed chloroplast *rps4*, mitochondrial *nad5* intron, and partial nuclear large ribosomal subunit sequences of 103 genera of Dicranidae and 20 outgroup taxa. Maximum likelihood and Bayesian analyses reveal strong support for many clades, however, few of these clades are reflected in the traditional taxonomy of the group. The Pottiaceae, Grimmiaceae s.l., and Calymperaceae are, in general, supported as phylogenetic groups, while others, such as the Dicranaceae, Ditrichaceae, and Rhabdoweisiaceae are polyphyletic. We contrast levels of molecular and taxic diversity within the Dicranidae.

13.6.7. *Physcomitrella patens* as a powerful model organism for EvoDevo studies: functional analysis of moss homologs of the floral regulator FLO/LFY

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Physcomitrella patens is an excellent moss model because of the feasibility of efficient gene targeting, which enables us to analyze gene functions of interest. To get insights into the evolution of flowers, the function of *PpLFY* genes, *P. patens* homologs of the floral regulator *FLO/LFY* genes, were analyzed. We generated disruptants of *PpLFY* genes, which hardly formed sporophytes though gametangia were normally differentiated. Confocal laser scanning microscopy demonstrated that fertilization occurred in *PpLFY* disruptants but zygote development was arrested at the single-cell stage. Crosses between the disruptants and wild-type strain supported these observations. Thus *PpLFY* genes regulate the first cell division of zygote and such a role is different from those of other *FLO/LFY* genes in angiosperms. The very rare sporophytes of the *PpLFY* disruptants showed mostly normal organogenesis, but had abnormalities in the pattern of cell division, suggesting *PpLFY* genes are necessary for proper cell division throughout sporophyte development. Other insights into the evolution of plant development using the *P. patens* model system are briefly reviewed.

13.7.1. At the edges of Cyperworld...

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Although our phylogenetic understanding of Cyperaceae continues to improve, attention is drawn to several unresolved morphological problems.

Mapanioids. Do we see spikelets or spikes or spicoids? And what about the plicate lateral "hypogynous scales", and the unseen oddities in *Chrysitrix*? Are there really terminal flowers in *Scirpodendron*? (Yes!) *Caricoids*. Might there be a morphological link from scirpoids to caricoids? (Yes!) *Fimbristyloids*. How to understand the adaxial "floral" scale in *Nelmesia*? *Cyperoids*. The laterally flattened ovary of *Pycneus*, *Kyllinga*, *Queenslandiella* - is it unique or a parallelism or a convergence? *Schoenoids*. The spikelet rachilla is a monopodium or a sympodium? And are the flowers thus lateral or terminal? (Well, ...) *Scleroids*. How can we link the (seemingly?) terminal flower in Bisboeckelereae with the pseudolateral ones in *Scleria*? What are the "complex" prophylls in Trilepideae? All these point to a brilliant future for morphological studies!

13.7.2. Phylogeny of Cyperaceae based on DNA sequence data: current progress and future prospects

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The family Cyperaceae is composed of 108 genera and c.5500 species and has an almost cosmopolitan distribution. Phylogenetic studies of the family, using DNA sequence data, have steadily increased over recent years. These have usually targeted particular groups within the family. At the Monocots III conference in 2003 a phylogeny of Cyperaceae based on *rbcL* data was presented. Since then further taxa have been sampled for *rbcL* and data are now available for a large majority of the genera. In addition a *trnL-F* dataset has been assembled, with representative

taxa from across the family. Analyses based on these datasets, run separately and in combination, will be presented with a discussion of subfamily and tribal recognition. Recent molecular work that has been carried on taxa in tribes Abildgaardieae, Arthrostryliadeae, Schoeneae and Scirpeae will be highlighted. Priorities for future work will be discussed.

13.7.3. Small is beautiful in Scirpeae

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The tribe Scirpeae comprises 11 genera. These genera are nowadays more or less well-defined, but this was not always the case. *Scirpus* of all genera has long been treated as a dustbin genus grouping Cyperaceae without clear diagnostic features. As better phylogenetic tools became available, the description of this genus became much more restricted. Still the affinity of some scirpoid species is not at all clear. From the Andes, a number of diminutive scirpoid species are known that are characterized by a single terminal spikelet or a small head of spikelets. An intensive study of the morphology, anatomy and molecular phylogeny resulted in the description of a new genus *Zameioscirpus*, a new circumscription of *Phylloscirpus* and a new species in *Oreobolopsis*. The minimal morphological differences between *Oreobolopsis* and *Trichophorum* and the results from a molecular analysis suggest that the species of *Oreobolopsis* should be recombined in *Trichophorum*, of which however more species from Eurasia should be studied first.

13.7.4. An overview of the Rhynchosporeae (Cyperaceae)

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The genera *Rhynchospora* (ca. 300 species) and *Pleurostachys* (ca. 32 species) form a distinct clade within the Cyperaceae and are most diverse in tropical America. While *Pleurostachys* is exclusively found in forests, *Rhynchospora* is ecologically diverse, occurring in savannas and forests as well as more specialized habitats such as black-water rivers and short-lived annuals. Kükenthal, in his 1949-51 monograph of *Rhynchospora*, recognized two subgenera, five supra-sectional taxa, and 29 sections; he recognized *Pleurostachys* as distinct from *Rhynchospora*. Current research includes revisions of *Pleurostachys* and *Rhynchospora* sections *Pluriflorae* and *Longirostres* as well as studies of anatomy and cytology. Preliminary phylogenetic analysis of *trnL-F* plastid gene suggests that *Pleurostachys* is distinct and that, within *Rhynchospora*, a large clade comprising much of subgenus *Haplostylis* is distinct. Subgenus *Rhynchospora*, however, and the remaining sections of subgenus *Haplostylis* are poorly resolved but show some evidence of clustering into clades resembling some of Kükenthal's supra-sectional groupings.

13.7.5. What is a genus in Cyperae?

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Cyperaeae comprises 900 species in 19 genera. *Cyperus* is the largest genus (550 species), while 13 of the genera have under five species. Most genera are diagnosed by few characters, causing difficulties in delimitation and varying generic concepts. Ongoing DNA studies support Cyperaeae as monophyletic and comprising the *Cyperus* and the *Ficinia* clades. The *Cyperus* clade, with predominantly distichous glume arrangement, is not monophyletic and two groups resolved differ in the presence or absence of Kranz anatomy, but with no defining morphological characters. The *Ficinia* clade has spherical glume arrangement, but some taxa with distichous glume arrangement have been included in *Cyperus*. *Hellimuthia*, previously included among the Mapanioideae due to presence of two floral scales, is resolved among the *Ficinia* clade. Oddly, *Scirpus ficinioides* and *S. falsus* are included in *Ficinia* clade, yet these taxa have bristle-like perianth. The DNA results reveal character evolutionary patterns, point to the need for revising tribal circumscription and support the

unpopular taxonomic view of merging the 15 genera belonging to the *Cyperus* clade into one genus.

13.7.6. Homology problems in cyperoid flowers: a floral ontogenetic approach.

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In Cyperaceae, two large subfamilies, Mapanioideae and Cyperoideae, can be distinguished, as suggested by recent molecular phylogenetic analysis (Simpson et al. 2004). A theoretical cyperoid flower consists of two whorls of each three perianth parts, a diplostemonous androecium, and a tricarpellate gynoecium. In all cyperoid taxa, however, there is a tendency to reduce the number and/or modify the shape of the floral parts. This has caused controversy about the interpretation of cyperoid inflorescences, flowers, and floral parts. We present SEM images of the floral ontogeny of *Scirpus*, *Eriophorum*, *Eleocharis*, *Fuirena*, *Dulichium*, *Cyperus*, *Scirpoides*, *Ficinia*, and *Schoenus*, showing that all flowers studied in Cyperoideae originate according to the same *Scirpus*-like ontogenetic pattern. Our results allow us also to understand the nature of the perianth parts in *Eriophorum* and *Fuirena*, and of the hypogynous stalk in *Ficinia*. Moreover, our results clearly show that in all the cyperoid genera studied, the spikelets consist of an indeterminate rachilla, with lateral empty or flower subtending glumes.

13.7.7. It's a flower, it's a spikelet: the guessing game in Cyperaceae - How do we resolve floral homology in the sedges?

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Uncertainty about flower and inflorescence homology exists across Cyperaceae. Of particular interest are homology of the flower, spikelet and associated bracts of Cyperaceae and similarly-named structures of Juncaceae. Principles that should guide investigation of cyperaceous reproductive homologies are that evidence should be considered within the broader context of phylogeny and floral homology of monocots and that multiple lines of evidence (corroboration) are necessary. Essential contributions are (1) comparative studies of spikelet development, as well as mature morphology, in diverse species; (2) mapping of characters onto robust phylogenies; and (3) use of gene expression techniques, as in the grasses. Because of their sister-group relationship to the rest of the family, as well as controversies about homology of their spikelets, mapaniids are a priority. We present our work on spikelets in this clade, esp. *Exocarya sclerioides* where inflorescence units appear monopodial.

13.8.1. Progress in Malpighiales phylogeny: new insights from eight genes

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Malpighiales include 29 families previously assigned to 13 different orders sensu Cronquist. While the monophyly of most families within the order has been established, interfamilial relationships are largely unclear. Our phylogenetic analyses of DNA sequence data from 8 genes (plastid *atpB*, *matK*, *ndhF*, and *rbcl*; nuclear 18S and *PHYC*; and mitochondrial *nad1B-C* and *matR*) spanning nearly 120 malpighiale taxa is beginning to resolve many of the major subclades within the order. These data further support the recent addition of Rafflesiaceae s.s. and *Centroplacus*, exclusion of Peridaceae, sister relations of Elatinaceae+Malpighiaceae and Putranjivaceae+Lophopyxidaceae, and inclusion of *Paradrypetes* in Rhizophoraceae s.l. New findings show Podostemaceae apparently nested within Hypericaceae, and a well-supported sister relationship between Phyllanthaceae and Picrodendraceae. The implications of these new results for the evolution of morphological characters, the problems of horizontal gene transfer, and for biome evolution will be discussed.

13.8.2. Phylogeny, character evolution, and classification of the Flacourtiaceae/Salicaceae complex

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Recent phylogenies drawn from DNA sequence data have affirmed the polyphyletic nature of the angiosperm family Flacourtiaceae, and a taxonomic rearrangement has been proposed which places most of the taxa into two major families, Achariaceae and Salicaceae. Presented here is a more robust phylogeny of one of these families, the Salicaceae *sensu lato*, which includes Salicaceae *sensu stricto*, the monotypic Scyphostegiaceae, and the majority of the former Flacourtiaceae (>80% of the species). Phylogenetic relationships were inferred from morphological data gathered for all genera and DNA sequence data gathered for 89% of the genera. A new classification is proposed based on this phylogeny that optimizes morphologically homogeneous groups. Samydaceae are resurrected as a family, Scyphostegiaceae are maintained as traditionally circumscribed, and a modified but more homogeneous Salicaceae, including part of the non-cyanogenic Flacourtiaceae, are introduced. *Ahernia* is moved to Salicaceae, Phyllobotryeae are moved to Achariaceae, and a new family near Malvales is created for *Gerrardina*.

13.8.3. Molecular phylogeny of Malpighiales and Euphorbiaceae, and evolution of ovule and seed coat characters

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Molecular phylogeny of Malpighiales and Euphorbiaceae is investigated to clarify relationships within the order and the family using sequence data from three plastid DNA *rbcL*, *atpB*, and *matK* and one nuclear 18S rDNA. Parsimony analyses of Malpighiales based on 106 genera in 24 families resulted in two most parsimonious trees. The monophyly of Euphorbiaceae and the other groups of families were well supported, and four genera (*Pera* etc.) with a tracheoidal exotegmen, which were previously placed in Acalyphoideae, diverged at the first branch within Euphorbiaceae. Using the four genera as an outgroup we conducted analyses of 74 genera of three subfamilies in Euphorbiaceae and obtained two most parsimonious trees. Their strict consensus tree showed that Suregada diverged at the first branch, followed by tribe Adenoclineae, the rest of Crotonoideae, the rest of Acalyphoideae, and Euphorbioideae. Both Euphorbioideae and Acalyphoideae (except for the four genera used as the outgroup) are monophyletic, and that Crotonoideae are paraphyletic. We also found that the major clades were supported well by anatomical evidence of ovule and seed coat.

13.8.4. Phylogeny and Evolution of the Passiflorineae

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The relationships among the families of Malpighiales are poorly resolved. One group of families, however, is consistently recovered in phylogenetic analyses based on chloroplast *ndhF* and mitochondrial *matR*, albeit poorly supported. This group encompasses the taxa with parietal placentation (plus Goupiaceae) in Malpighiales and is referred to as suborder Passiflorineae. Presented here is a phylogeny of Passiflorineae at the generic level based on both morphological and molecular data sets. Like previous phylogenies, this analysis indicates a close relationship between Passifloraceae, Malesherbiaceae, and Turneraceae and between Samydaceae, Scyphostegiaceae, and Salicaceae. It also indicates a close relationship between Achariaceae *sensu lato* (including the cyanogenic part of the former Flacourtiaceae) and Violaceae. The distribution of character states for cyanogenic glucosides, anther dehiscence, and wood anatomy corresponds well with this phylogeny. The monophyly of Achariaceae *sensu lato* is addressed, as well as the evolution of corona, stamen number, and sexual systems.

13.8.5. Wood evolution in the novel eudicot order Malpighiales

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The Malpighiales represents “a bewildering assemblage of highly specialised groups as well as many primitive elements” according to Chase *et al.* (2002). This variety is reflected in their wide range of habit and habitats and diverse wood anatomy. Here we review new and published information on the wood anatomy of Malpighiales, particularly the important timber families Chrysobalanaceae, Clusiaceae, Euphorbiaceae, “Flacourtiaceae” and Salicaceae. Some families (e.g. Malpighiaceae and Passifloraceae) include lianas with anomalous secondary growth. Other characters with phylogenetic potential include vessel distribution, vessel perforation plate type (simple, scalariform or both types), vested pits, vessel-ray pitting, axial parenchyma patterns, ray structure and type of cell inclusions (silica bodies, calcium oxalate crystals). We evaluate wood evolution in Malpighiales by plotting some of these wood characters onto recent molecular cladograms at the order and family levels.

13.8.6. Phylogeny of the flaxes: Evolutionary relationships in the Linaceae and *Linum*.

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Linaceae, best known for the useful fibers and oils from the cultivated flax, *Linum usitatissimum*, is a widespread family with 13 genera and 300 species, ranging from diminutive temperate annuals to woody tropical trees and climbers. The position of Linaceae in Malpighiales has been clarified in large-scale phylogenetic analyses, but relationships within the family have not been addressed. To determine the circumscription of monophyletic Linaceae and the relationships of the large, cosmopolitan genus *Linum* to the other temperate genera, especially the segregates from *Linum*, we sampled all 13 Linaceae genera and the 5 sections of *Linum*. Phylogenetic analyses of *rbcL* sequences in the context of the Malpighiales show that the tropical genera of Linaceae subfamily Hugonioideae subtend the monophyletic temperate subfamily Linoideae. Within Linoideae, analyses of *trnK* 3' and *trnL* introns, *trnL*-F spacer, and nuclear ITS show that *Linum* is not monophyletic: the blue-flowered linums are sister to the yellow-flowered linums, *Hesperolinon*, *Sclerolinon*, *Radiola* and *Cliococca*. The central Asian *Tirpitzia*, *Reinwardtia*, and *Anisadenia* are basal in the Linoideae.

13.8.7. Phylogeny and character evolution in Clusiaceae and their immediate relatives.

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Three subfamilies of Clusiaceae have been recognised based on their morphology. After extensive sampling, molecular data have been used to evaluate their status and relationships, and also those of Podostemaceae and Bonnetiaceae. The monophyletic Clusioideae and Kielmeyeroideae are likely sister taxa. Molecular data show that Hypericoideae, or part of them, are sister to the aquatic Podostemaceae. The morphological evolution of this complex is studied in detail using a data base of over 125 characters. Within Clusioideae, the monophyly of the Neotropical Clusiaceae, with arillate seeds and a non-fasciculate androecium, has strong support. The Pantropical Garcinieae and Symphonieae are close molecularly, yet very different in androecium and stigma structure. The Pantropical Kielmeyeroideae show notable variation in embryo morphology and oil/resin-secreting anther glands. Stigmatic variation is of particular interest in Hypericoideae, Bonnetiaceae and Podostemaceae. Germination, seedling morphology, leaf margin, and position of cork cambium in both stem and root, are among other character systems whose evolution is discussed.

13.9.1. Phylogenetic structure of Poaceae (R. Br.) Barnhart subfamily Pooideae Benth. based on sequence data of four chloroplast-encoded genes (*matK*, *ndhF*, *ndhH*, and *rbcl*)

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Pooideae phylogenetic structure was evaluated by cladistic analysis of over 100 genera of Pooideae and ca. 40 outgroups. With taxa from all tribes of Pooideae and several unusual genera sampled for the first time, a robust structure for relationships among related subfamilies and tribes is as follows: Ehrhartoideae (Bambusoideae Pooideae), and within Pooideae; Brachyelytreae ((Lygeae Nardeae) (Phaenospemateae (Stipeae ((Brylkinieae Meliceae) (Diarrheneae (Brachypodieae ((*Littledalea* (Bromeae Triticeae))(Aveneae Poeae). Asian genera, *Duthiea* and *Sinchoasea*, formerly Aveneae or subfam. Arundinoideae s.l., align in a clade with the Asian *Phaenospemata*, and the Australian *Anisopogon*. *Ampelodesmos* aligns among sampled Stipeae, including: *Trikeria*, *Hesperostipa*, *Oryzopsis* s.s., *Nassella*, and *Timouria*. *Psammochloa*, hitherto Stipeae, aligns with *Bromus*, but *Littledalea* does not. Poeae s.l. includes 2 cpDNA clades corresponding in part to Aveneae and Poeae, but several lineages are switched, and Hainardieae are included within Poeae s.s. Pooideae classification is revised, and morphological and biogeographic patterns are considered.

13.9.2. Evolutionary analysis of the Poaceae subfamily Pooideae tribal complex Aveneae-Poeae: systematic and biogeographic implications

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Phylogenetic analysis of the sister tribes Aveneae and Poeae has clarified the evolutionary boundaries of their major lineages and their interconnections. Sampling included a large generic representation of Poeae (75%) and Aveneae (67%). Parsimony and Bayesian reconstructions based on ITS and *trnT* sequences recover a similar topology, distinguishing: i) a Loliinae s. l. + close allies clade, where the main genus *Festuca* is paraphyletic diverging into a grade of basal broad-leaved *Festuca* lineages and a supported clade of recently evolved fine-leaved *Festuca* lineages; ii) a Poinae s. l. clade, where the main genus *Poa* is monophyletic if other minor genera are included within it and if subgen. *Andinae* is removed; iii) an Aveneae core clade, formed by the most recently evolved sister clades Koelerinae /Aveninae and Agrostidinae, plus a series of other less related lineages. Most reconstructions recover a closer relationship of Poinae to Aveneae than to its cotribal group Loliinae though these relationships are weakly supported. Separating the Aveneae and Poinae core clades is a series of intermediate lineages including a mixed group of Aveneae + Poeae taxa.

13.9.3. Reticulate evolution and origins of northern hemisphere allopolyploid *Elymus* (Triticeae)

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The wheat tribe, Triticeae, is recognized for its complex evolutionary history. Its reticulate history is implicitly demonstrated by extensive conflict among diploid gene trees, and explicitly confirmed by the presence of numerous allopolyploid combinations. *Elymus* is a large, widespread, entirely allopolyploid genus that includes at least one *Pseudoroegneria* genome, combined with genomes from one or more of several other genera from the tribe. Molecular phylogenetic analyses have been useful for clarifying the evolutionary origins of *Elymus* species, with data from most of the tetraploids usually confirming cytogenetic hypotheses of origins. Data from multiple chloroplast and nuclear genes suggest that the origin of hexaploid *Elymus repens* is more complex, possibly combining (1) allopolyploidy involving *Hordeum*, *Pseudoroegneria*, and an unknown donor, and (2) introgression from *Taeniatherum* and a species from outside of the tribe.

13.9.4. Incongruence and the phylogeny of the Triticeae (Poaceae)

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The Triticeae includes some of the world's most important cereals, wheat (*Triticum*), barley (*Hordeum*), and rye (*Secale*), plus a wide variety of important forage grasses. Despite its economic importance the phylogeny of the tribe remains largely unsettled. The vast majority of the species are allopolyploids, with an apparently known history, but the relationships of their diploid progenitors are obscure. Nearly all phylogenies of the Triticeae (Poaceae), which have been based on more than a single data set, show a high level of incongruence between different data partitions. The contribution of individual data sets from different genome compartments are explored in this analysis based on eight data sets, viz. nucleotide sequences from three plastid genes (*rbcl*, *ndhF*, and *rpoA*), one mitochondrial gene (*cox1l*), two single-copy nuclear genes (DMC1 and EF-G), one multi-copy nuclear region (ITS), and morphology.

13.9.5. Phylogeny and biogeography of Bromeae

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Tribe Bromeae (Poaceae: Pooideae) comprises a single genus, *Bromus*, with ~160 species distributed widely in temperate regions. Phylogenetic analyses of nuclear (rDNA internal transcribed spacers) and plastid (*trnL* intron and *ndhF*) data identified several lineages with weak to robust support, some corresponding to traditionally recognized taxa (e.g., sects. *Bromus* and *Genea*). However, there is some evidence of incongruence between the nuclear ribosomal region and the plastid genome. In addition to augmenting our original taxon sampling, we are surveying additional plastid loci and the external transcribed spacer (ETS) region of nuclear rDNA to obtain better resolved and supported trees from these linkage groups, as a first step in clarifying any intergenomic conflict. Several major clades are supported by unambiguous indels; one in the *trnT-trnL* spacer, for example, supports a clade of some North American taxa in *Bromopsis*, a section with little plastid DNA sequence variation. We also provide an overview of how the new phylogenetic data impact upon our understanding of the biogeography and morphological diversity of the major lineages of *Bromus*.

13.9.6. Speciation in *Hordeum* (Triticeae): The interrelation of biogeography and ecology

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The barley genus *Hordeum* L. occurs with 31 species in the meridional and temperate regions of the northern hemisphere, South America, and South Africa. The center of diversity of the genus is southern South America where ten diploid and six polyploid species occur. Phylogenetic analysis of three nuclear loci revealed a rapid and relatively recent radiation within this area during the last 4-2 million years. The combination of the nuclear phylogeny with genealogical data of chloroplast haplotypes resulted in the resolution of repeat long-distance dispersals and major vicariance events as mechanisms contributing to allopatric speciation in this group. Ecological differentiation took place within and between two major South American species groups. In these groups species possess different habitat preferences with regard to humidity, salinity, and elevation, supporting the view of ecological niche differentiation. The Eurasian species groups were affected by far reaching extinctions during the Pleistocene, which aggravated the differences in *Hordeum* species numbers between Old and New World.

13.9.7. Biogeography and classification of New World Pooideae (Poaceae)

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Of the 3851 native species of grasses in the New World, 1340 are members of the Pooideae. In our classification of the Pooideae we recognize eight tribes, 17 subtribes and 83 genera as native to the New World. Over 60% of the Pooideae belong to the Poeae tribe (827 species), including four of the five largest genera: *Festuca*

(209), *Poa* (175), *Calamagrostis* (132), and *Agrostis* (67). The other, *Nassella* (Stipeae) has 115 species. The Pooideae is the largest subfamily of grasses in the New World with less than 2% of these species being pervasive. Argentina (476 species/122 endemics) and the USA (432/135) have the richest Pooideae flora, followed by Chile (296/54), Canada (246/7), Peru (230/66), Mexico (211/71), and Bolivia (207/23). The Pooideae represent over 70% of the grass flora of Canada and Chile, as opposed to approximately 45% for Argentina and the USA. The subfamily is completely or nearly absent from Belize, El Salvador, Nicaragua, and the Guiana Shield. High species diversity that is mutually exclusive occurs in temperate to subtropical regions of the USA in North America and in the pampas region of Argentina, Paraguay, Uruguay, and Brazil in South America.

13.10.1. Alpine plant reproductive biology: the emerging picture

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In alpine communities above treeline, increasingly colder temperatures are paralleled by altitudinal changes in pollinator composition and decreases in flower visitation rates. We will evaluate the advances made, and gaps in knowledge required to determine whether alpine floras exhibit fundamental differences in: breeding systems, pollination mechanisms, levels of genetic variation and floral characteristics, in comparison with their lowland counterparts. While obligately outbreeding systems decrease in frequency with elevation on some mountains, the reverse trend has also been found. Some alpine species are strong selfers and have limited genetic variation whereas others show considerable genetic variation indicative of significant outcrossing. Generalist and specialist pollination systems can co-occur in the alpine, but broad comparisons show that connectance is not necessarily high. Increased flower longevity at higher elevations seems to be common trend - it might not only allow compensation for low pollination rates, but also explain low pollinator density per se. Research financed by: FONDECYT 1020956, 7020956, ICM P02-051

13.10.2. Is there a typical alpine flower?

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Flowers of alpine species are often said to be more brightly coloured and larger than flowers of low-land species, the proposed reason being that alpine plants must be particularly showy to attract the few pollinator individuals that exist in alpine habitats. Moreover, alpine species are believed to have structurally simpler flowers that can be effectively pollinated by a wide array of pollinators. However, in reality we know little about how floral traits of alpine species differ from those of species occurring in other habitats.

We examined if alpine species have special flowers by comparing flower size, colour and morphology of alpine, coastal and south-eastern species in Norway.

With the exception of one notable floral feature, there were no indications suggesting that there exist a typical alpine flower, at least in Norway. In general, floral traits of alpine species could be special if a species filtering process based on floral traits occurred *before* species migrated to the alpine, or if selection acted strongly on floral traits *after* migration to the alpine. Apparently, none of these two processes have occurred.

13.10.3. Variations in flowering phenology in alpine ecosystem: its importance as a selective force

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Flowering schedule of alpine plants is basically determined by climatic conditions and snowmelt regimes at micro scale. Spatiotemporal variations in flowering timing and duration of individual species strongly influence the reproductive situations. Flowering in early season often results in pollen limitation due to low activity of pollinators under cool conditions. Late flowering, whereas, results in failure of seed set due to limited season length. Segregation of flowering season may isolate the gene flow through

pollination process among adjacent local patches, resulting in a fragmented or genetically differentiated structure of populations within local areas. At community level, a snowmelt gradient creates a complex and diverse flowering structure. This may be beneficial for flower visiting insects, but it may cause severe pollination competition among co-flowering plants. Thus, patterns of flowering phenology act as an important selective force on alpine plants.

13.10.4. Adaptive peaks or evolutionary traps: are alpine plants victims of their own success?

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Brief growing seasons, cold temperatures, and high winds prevail above timberline. Alpine plants have traits that allow them to flourish despite these limitations. I use two examples to argue that such traits will reduce the capacity of alpine plants to tolerate aspects of global change. 1. Flower heliotropism is mainly limited to plants of alpine and arctic habitats. Heliotropism in the snow buttercup, *Ranunculus adoneus*, has a favorable impact on flower carbon balance and seed growth under current alpine conditions. However, when ambient temperature is increased experimentally, heliotropism exacts a carbon cost in respiration compared to stationary controls. Results suggest that solar tracking will have a net energetic cost under warmer conditions. 2. Alpine plants typically are dwarfed in stature. Low stature is thought to resist lodging under high winds above treeline. Seed bearing stalks are shorter in the alpine dandelion, *Taraxacum ceratophorum* than in its exotic congener *T. officinale*. Because seed dispersal in dandelions depends on height, exotics have an advantage over alpine natives in colonization of open disturbances and may replace them over time.

13.10.5. Collective sustaining of pollinators by globeflowers

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Cooperation among unrelated individuals challenges the individualistic-based Darwinian view of evolution, which predicts that free-riders benefiting from the common resource without paying its cost should invade. Globeflowers are exclusively pollinated by flies whose larvae feed on their seeds. Although all plants benefit from mutual cooperation to sustain the pollinator population, a cheating individual could do even better by exploiting the cooperative efforts of others, without paying the price of rearing pollinator larvae. Here we document individual versus population conflict in globeflowers in sustaining their pollinating flies throughout the range of this arctic-alpine European plant. Despite considerable variability in ecological conditions and pollinator densities across populations, the percentage of seeds lost to pollinators remains strikingly stable over time and space. The stability of the interaction relies on density-dependent competition among larvae co-developing in flower heads.

13.10.6. Insectivorous carnivores negatively affect pollinator visitation and reproductive output in alpine plants.

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Studies on plant-pollinator interactions have largely neglected the potentially negative effects of predators of pollinators on seed output. Pollinator anti-predatory behavior may affect flower visitation patterns, pollen transfer, and plant reproduction. I discuss experimental evidence showing lizards to reduce pollinator visitation and seed output in an Andean shrub. The lower alpine belt holds a high density of lizards and low shrubs interspersed among lizard-inhabited rocks. Lizard exclusion resulted in a 2-4 fold enhancement of the visitation rates of the most frequent pollinators of this shrub, the duration of visits, and seed output. In a natural experiment, fly visits were 9 times shorter, and visitation rates and seed output were 2-3 times lower on shrubs adjacent to lizard-occupied rocks compared to those distant from rocks. Our results show lizards to alter pollinator behavior and elicit strong top-down negative effects on seed output. Such effects may be especially important in the alpine, where pollinator activity can be low, and pollen limitation occurs.

13.10.7. Seed banks and seed germination in the alpine zone**B. Erschbamer;**

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The investigation of seed banks, seed persistence, and germination strategies becomes more and more important as these traits are essential for the explanation of life histories and ecosystem functioning and probably also for the evaluation of climate change impacts in the future. During the last decades, a considerable amount of papers showed that seed banks exist in alpine soils, being important drivers of regeneration. The aims of the present contribution are (1) to highlight the importance of alpine seed banks, (2) to describe short and long term persistence of alpine seeds, (3) to answer the question if seed size, shape and mass are important in determining the alpine seed bank behaviour, and (4) to compare seed banks and germination strategies along primary succession. Data from the central alpine glacier foreland of the Rotmoosferner (Ötztal, Tyrol, Austria) are shown. It was suggested that early colonizers - in contrast to later successional species - will not build a seed bank. This hypothesis can be rejected for the study site in the Central Alps.

13.11.1. Seasonal adjustments in photosynthesis and photoprotection**W. W. Adams III, C. R. Zarter, V. Ebbert, K. E. Mueh, V. S. E. Amiard, B. Demmig-Adams;**

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Overwintering herbaceous species typically maintain fully functional photosynthetic electron transport systems and rely primarily on flexible zeaxanthin-dependent thermal energy dissipation for photoprotection. In contrast, many evergreens exhibit prolonged and pronounced reductions in photosynthetic electron transport capacity during winter, coupled with sustained high levels of zeaxanthin-dependent thermal energy dissipation. Under more extreme conditions at higher elevations, all major evergreen components of the Rocky Mountain subalpine ecosystem (conifers and a broad-leaved ground cover) exhibited strong and lasting photosynthetic downregulation. During the vernal upregulation of photosynthesis, both photosynthetic capacity and the degree of sustained energy dissipation varied from day to day with changing environmental conditions. The role of particular proteins in these seasonal adjustments, including D1, PsbS in flexible dissipation, and Elips and/or Hlips in sustained dissipation, will be examined. Differences in photosynthetic adjustment between apoplastic and symplastic loaders will also be considered.

13.11.2. Wintertime influences on net ecosystem CO₂ exchange in a high-elevation, subalpine forest**R. K. Monson;**

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Wintertime conditions affect the exchange of CO₂ between ecosystems and the atmosphere through influences on tree photosynthesis and soil respiration. Although wintertime photosynthesis is downregulated, wintertime soil respiration continues at a relatively high rate, resulting in the loss of up to 60% of the carbon sequestered by the ecosystem the previous summer. Winter soil respiration rates are dependent on the depth of the late-winter snow pack, which in turn influences beneath-snow soil temperatures. Wintertime loss of sucrose from plant roots, perhaps due to mechanical disruption during soil freezing, supplies carbon substrate for microbial activity and soil respiration. During the spring snowmelt forest net CO₂ uptake reaches its maximum annual rate, due to the combination of warm air temperatures, which promote high rates of photosynthesis, and low soil temperatures, which promote low rates of soil respiration. The evergreen growth form allows subalpine conifers a "quick start" to photosynthesis at the end of winter; permitting them to minimize the developmental constraint to seasonal photosynthesis that is exhibited by deciduous trees.

13.11.3. Snow-induced hypoxia - a keynote towards the descent of birch treelines in snowy regions**D. Gansert;**

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On snowy mountains in humid northeastern Asia the descent of the birch treeline by 800 m below the thermal upper distribution limit is discussed in terms of a synergistic effect of oxygen and carbohydrate deficiency during bud break. In spring, hypoxia under compressed snow hampers oxidative respiration and thus, causes ATP deficiency in the parenchymatous tissues of the wood-body. The impact of hypoxia on tree survival will increase when most reserve carbohydrates have been consumed so that enhanced ATP demand of live tissues during bud break cannot be further compensated. Thus, on condition of prolonged snow cover deciduous treelines may occur where metabolic compensation of hypoxia limits anabolism during the early season. This hypothesis of hypoxia-induced carbohydrate limitation regards oxygen supply to the woody corium as a key factor for the establishment of birch treelines in snowy, periodically water-logged subalpine and subarctic regions. Measurements of dissolved O₂ in the sapwood of birch proved that oxygen deficiency increased from bud break until frondescence, when a deficit of nearly 90% of air saturation marked a maximum of oxygen depletion.

13.11.4. Physics, physiology, and ecology of wintertime xylem cavitation**J. S. Sperry, J. Pittermann;**

University of Utah, Salt Lake City, UT, United States.

Cavitation occurs in response to freeze-thaw cycles and water stress, and both contribute to potentially high blockage of xylem transport and winter-related dieback. Several adaptations minimize the problem. Avoidance of cavitation requires narrow xylem conduits and air-tight inter-conduit pitting. This "strategy" functions equally well for conifer tracheids or angiosperm vessels but results in inefficient water conduction under favorable conditions. Alternatively, wide and efficient conduits that cavitate in winter can be refilled or replaced prior to the growth season. This strategy requires any evergreen foliage to be resistant to water loss to avoid severe stress. The refilling occurs by root pressure in many woody angiosperms, and by an unknown mechanism in conifers. Impairment of refilling can lead to dieback. Replacement of non-refilled conduits occurs in ring-porous trees but requires late leafing and a shorter growing season. Although the basic mechanisms of freeze-thaw related cavitation and refilling are known, important gaps remain.

13.11.5. JrSUT1, a putative xylem sucrose transporter, is up-regulated by freezing temperatures over the autumn-winter period in walnut tree (*Juglans regia* L.)**M. Decourteix¹, G. Alves¹, N. Brunel¹, T. Améglio², A. Guilliot¹, M. Poirier², M. Bonhomme², R. Rageau², G. Pétel¹, S. Sakr¹;**¹Université Blaise Pascal, Aubière, France, ²INRA, Aubière, France.

Sucrose has long been reported to play multiple roles in the winter biology of temperate woody species. However, no report on the molecular basis of sucrose transport has yet been made. In walnut tree, we showed that active absorption of sucrose from xylem vessel to vessel-associated cells is higher when samplings are done after a freezing period. We tested whether this increased sucrose influx is the result of the sucrose transporter regulation in xylem tissues.

A putative sucrose transporter cDNA (*JrSUT1*: *Juglans regia* sucrose transporter 1) was cloned from a xylem-derived cDNA library. Over the autumn-winter period, *JrSUT1* transcripts and respective proteins were present in parenchyma cells. They were specifically and highly detected when samplings were preceded by freezing/non-freezing period. The up-regulation of *JrSUT1* level was also found in controlled conditions. Immunolocalization studies showed that JrSUT1 and JrAHA were co-localized to vessel associated cells, which control solute exchanges between parenchyma cells and xylem vessels. These data are discussed with respect to the winter biology of walnut tree.

13.11.6. Seasonal changes in carbon and nitrogen metabolism in *Spartina alterniflora***R. J. Ireland, L. Adongo;**

Mount Allison University, Sackville, NB, Canada.

The intertidal zone of many Eastern North American salt marshes is often dominated by the halophytic perennial grass, *Spartina alterniflora*. Over winter, this plant stores nutrient reserves in underground rhizomes, which become very metabolically active in spring and fall, when they are exporting organic nitrogen and carbon to the developing shoots. Much of the transported material

is in the form of asparagine, most of which is hydrolyzed to aspartate and ammonia by the enzyme, asparaginase in the young shoots. The aspartate has many fates, including transamination to other amino acids. We have isolated and purified five forms of aspartate aminotransferase from *Spartina* leaves, which show distinct seasonal activity profiles and substrate specificities. The ammonia is assimilated by glutamine synthetase, which is present as two forms in *Spartina* leaves, which also show distinct seasonal activity profiles. Antibodies specific to the two forms of GS revealed a complex pattern of changes in cellular distribution of the cytosolic and chloroplastic forms of GS during the spring and summer.

13.11.7. *Arabidopsis* vernalization requirement and response

C. Dean;

John Innes Centre, Norwich, United Kingdom.

Many plant species will not flower until they have experienced a long period of cold temperature, or 'winter'. This process, known as vernalization, ensures plants overwinter vegetatively and flower in the favourable conditions of spring.

Vernalization epigenetically regulates expression of a floral repressor, *FLC*. Prolonged cold results in reduction in *FLC* RNA levels, which then remain constant at that lower level when plants are moved back to warm temperatures. Identification of mutants defective in vernalization response has defined *VRN* genes required to cause down-regulation of *FLC* and maintain the cellular memory of vernalization.

Vernalization antagonizes the function of *FRIGIDA*, which up-regulates *FLC*. These combined activities thus prevent flowering until winter has passed. In contrast, vernalization works in parallel to genes such as *FCA* and *FY* to repress *FLC* expression.

The talk will address how these pathways interact to regulate *FLC* expression at different stages in the plant life-cycle and how these have changed in natural *Arabidopsis* variants adapted to very different growth conditions.

13.12.1. Tannin-containing plants for animal nutrition and health

I. Mueller-Harvey;

University of Reading, Reading, United Kingdom.

Tannins in animal feeds can produce useful benefits: better protein utilisation, growth rates, milk yields, fertility and improved animal health (prevention of bloat, reduction of intestinal parasites, treatment against diarrhoea). The classification into condensed or hydrolysable tannins has not proved useful in animal nutrition. Procyanidins and prodelphinidins in *Lotus*, *Onobrychis* or *Calliandra* sp. and ellagitannins in chestnut are beneficial, but proflisetinidins in *Schinopsis* sp., procyanidins in sorghum and complex mixtures of tannins in *Quercus* and *Terminalia* sp. are harmful or even toxic to ruminants. Moreover, slight changes in tannin structures can produce measurable effects. Unfortunately, it is not yet possible to predict which tannins produce what effects. Novel assays will be described that may prove more useful for relating laboratory measurements to animal performance. The inter- and intra species variation of tannins present opportunities for future applications of tannin-containing plants.

13.12.2. Importance of different phenolic compounds can be judged by inhibition of phenylalanine ammonia lyase, in seedlings of *Betula pubescens*

S. Keski-Saari, M. Falck, R. Julkunen-Tiitto;
University of Joensuu, Joensuu, Finland.

We studied carbon allocation patterns between plant growth and phenolic metabolism in birch seedlings at the beginning of their growth by inhibition of phenylalanine ammonia lyase (PAL), the first committed enzyme of phenolic metabolism. Seeds of *Betula pubescens* (Ehrh.) were germinated in inhibitor-free agar media and, at cotyledon stage, the seedlings were transferred into hydroponic culture with 2-aminoindan-2-phosphonic acid (AIP) in the growing medium. Three concentrations (5, 15 and 30 μ M) plus control were used. We have an opportunity to judge the importance of different phenolic compounds for the seedlings, since all phenolics were not equally inhibited by AIP. Flavonol glycosides were generally not affected by AIP and even increased in the leaves. In contrast, soluble condensed tannins strongly decreased in all organs, even with 5 μ M AIP. However, insoluble condensed tannins were only slightly inhibited. Our results suggest that flavonol glycosides, and bound tannins, have an important role

in the development of birch seedlings. In addition, AIP strongly decreased the growth of the seedlings and possible reasons for this will be discussed.

13.12.3. Profiling phenolic acids from hairy root cultures of *Daucus carota* L. for investigating p-hydroxybenzoate biosynthesis

A. Mitra, D. Sircar;

Indian Institute of Technology Kharagpur, Kharagpur, India.

Hairy root cultures of *Daucus carota* have been used to study the biosynthesis of p-hydroxybenzoic acid (p-HBA). This phenolic compound finds its application in food, medicine and cosmetics. Biosynthesis of p-HBA although demonstrated to proceed via phenylpropanoid pathway, conflict still remains on the chain-cleavage mechanism. In earlier years, attempts had been made to study p-HBA biosynthesis in carrot using elicited cell cultures, but failed to demonstrate the chain-shortening enzyme activity. Metabolite profiling of phenolics by HPLC/ESI-MS from these hairy roots showed a considerable high amount of p-HBA accumulation both in cytosol and in cell wall. Detection of a significant amount of soluble p-HBA in carrot hairy root is interesting, since this may permit us to go forward in discovering the phenylpropanoid chain-shortening enzyme. In fact, our preliminary findings on this enzymatic cleavage suggest that p-HBA biosynthesis is CoA independent and non-oxidative demonstrating stable p-hydroxybenzaldehyde formation in cell-free extracts. This aldehyde later converted into p-HBA. Work is being continued to confirm this hypothesis.

13.12.4. Hinokinin biosynthesis in *Linum strictum* ssp. *corymbulosum*

Ü. Bayindir¹, T. Umezawa², R. Sjöholm³, W. Alfermann¹, E. Fuss¹;

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Lignans are dimers of phenylpropanoid units. Recently the lignan (-)-hinokinin (HINO) was found in root and callus cultures of *L. strictum* ssp. *corymbulosum* (Mohagheghzadeh et al., in prep.). We established cell suspension and hairy root cultures of this plant in order to elucidate the biosynthetic pathway leading to HINO. Two pathways are under investigation: If matairesinol is a central intermediate, HINO can be formed via haplomyrfofin or pluviatolide, respectively. If the formation of the methylenedioxy bridges occurs earlier by using pinosresinol as substrate, sesamin instead of matairesinol could be an intermediate in HINO biosynthesis. We try to clone a cDNA encoding pinosresinol-lariciresinol reductase (PLR) which will be used in antisense experiments to check the responsiveness of PLR in HINO biosynthesis. A grant to Ürün Bayindir from "Turkish Higher Education Council" is gratefully acknowledged.

13.12.5. Molecular cloning and functional expression of benzoate:CoA ligase from *Sorbus aucuparia* cell cultures

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Institut für Pharmazeutische Biologie, Braunschweig, Germany.

Benzoic acids are precursors and signal molecules. In cell cultures of *Sorbus aucuparia* and *Hypericum androsaemum*, benzoic acid is a precursor of biphenyl and xanthone biosyntheses, respectively. Benzoic acid formation in *H. androsaemum* proceeds via cinnamic acid, with the underlying mechanism being CoA-dependent and non- β -oxidative. Three CoA ligases with different substrate specificities were detected. Cinnamate:CoA ligase channels cinnamic acid from the general phenylpropanoid pathway into the benzoic acid biosynthetic route. 4-Coumarate:CoA ligase is a well-known component of the general phenylpropanoid pathway. Benzoate:CoA ligase provides the starter substrate for benzophenone synthase and biphenyl synthase. Benzoate:CoA ligase from yeast-extract-treated cell cultures of *S. aucuparia* has been cloned, functionally expressed in *E. coli* and purified by affinity chromatography. The enzyme does not accept 4-coumaric acid and cinnamic acid as substrates.

13.12.6. The different possibilities of introducing hydroxyl groups in the flavonoid pathway

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Compounds belonging to the widespread group of flavonoids show a number of hydroxyl groups within their flavonoid structure. The most common flavonoids show a basic 5,7-hydroxylation pattern in the A-ring and a hydroxyl group in position 4' of ring B, which derive from the precursors in the formation of the C15-skeleton, malonyl-CoA and a suitable hydroxycinnamic acid-CoA ester. Further hydroxyl groups may be introduced in rings A, B and C by hydroxylating enzymes. There are two main classes of enzymes responsible for the introduction of hydroxyl groups in the flavonoid pathway, 2-oxoglutarate dependent dioxygenases and cytochrome P450 dependent monooxygenases. An overview is given on well-known enzymes (flavanone 3-hydroxylase, flavonoid 3'-hydroxylase and chalcone 3-hydroxylase) and on novel enzymes which recently could be demonstrated for the first time (flavonol 6-hydroxylase, flavonoid 8-hydroxylase).

13.12.7. Growth promoting nitrogen nutrition affects flavonoid biosynthesis of young apple (*Malus domestica* Borkh.) leaves

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Increasing nitrogen (N) nutrition of apple trees enhances shoot growth but depresses the concentration of flavonoids in young leaves. In the cultivar 'Golden Delicious', which is susceptible to the scab disease, the reduced amount of flavonoids is related to increasing susceptibility after high N nutrition. Several hypotheses exist which try to explain the trade-off between primary and defense-related secondary metabolism by a competition on common substrates, but nothing is known about the regulation at the enzyme level. A set of experiments was performed to elucidate the effect of N nutrition on the activities of key enzymes of the flavonoid biosynthesis and on the accumulation of hydroxycinnamic acids and different flavonoid compounds. The inhibition of flavonoid accumulation by high N nutrition could be confirmed, but the influence of N supply on the flavonoid enzymes was not evident. However, PAL activity seems to be downregulated, thus forming a bottleneck resulting in a generally decreased flavonoid formation. It could furthermore be found that the response of the scab resistant cultivar 'Rewena' to high N nutrition was not as strong as that of the susceptible cultivar 'Golden Delicious'.

13.13.1. Diversity of grasslands under pressure from environmental change

J. Fuhrer, A. Lüscher;
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Grasslands play an important role for agriculture and conservation, with their species richness reflecting local management and environmental conditions. In intensively managed, productive systems the influence of management dominates. In more diverse semi-natural systems, environmental drivers are more important, but maintenance of their diversity still requires some management, as either intensification or abandonment reduce diversity. Species diversity of semi-natural grasslands can be altered by changes in atmospheric conditions, including increasing pollution in the form of deposition of reactive nitrogen or ozone (O₃), carbon dioxide (CO₂), and global warming. Spatial and temporal patterns in these drivers are well documented, and data from long-term observations and from manipulative experiments are emerging that demonstrate their potential influence. Results will be presented and discussed in the context of possible changes in management to maintain grassland diversity, and the relationship between observed changes in grasslands and various drivers including management will be explored with data from a case study in the Swiss Alps.

13.13.2. Development of the xerotherm grassland vegetation of the military area Doupov over the past 30 years (western Bohemia).

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Military training area Doupov in the western part of the Czech Republic represents a unique landscape, mainly due to the continual absence of inorganic fertilising and preserved water

retention of deforested plots. A small portion of this area is subjected to a long-term management practices, featuring by their low intensity. Habitats of subxerotherm grasslands, which now belong to a highly endangered vegetation in cultural mainland, were well developed here. Their diversity and species richness is partly caused by the mineral rich volcanic rocks of the Doupov area. The abandonment of land, where competitive plants (apophytes) become to dominate, is now hazardous for biodiversity. On the other hand, successional trend leads to rich shrub communities, massively covering the most of this area. The overall number of grassland phytocoenoses has declined, floristic composition of vegetation has changed over the past 30 years. Preservation of various unique plant communities, often characterised by the strong development of endangered species, is necessary through continuous disturbance and/or keeping the water regime of stands.

13.13.3. Grasslands in changing landscapes: linking soil seed banks and habitat characteristics

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As shown in previous studies, in changing landscapes with increasing numbers of extensively managed grassland stands, the diversity between the respective vegetation is clearly reflected by the diversity of habitat characteristics including age. Little is known if the soil seed banks of these grasslands and their site characteristics may be linked in an analogous manner.

We therefore investigated the seed banks of 22 grassland stands, and their respective established vegetation and site characteristics. All stands were located in the Lahn-Dill-Highlands (Hesse, Germany). Data were analysed by means of ordination techniques (DCA and partial CCA), Mantel-tests, and ANOVA.

The composition of the established vegetation proved to be the most important variable influencing the composition and structure of the seed banks. However, soil chemistry (pH value and plant available phosphorus) and stand age explained additional proportions of the variance explained in the ordination model.

13.13.4. Niche complementary for nitrogen use - an explanation for the biodiversity and ecosystem functioning relationship in grasslands?

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The relationship between plant diversity and productivity has largely been attributed to niche complementarity, suggesting that an increasing number of species results in a more efficient resource exploitation. We tested this hypothesis with respect to temporal, chemical and spatial N-uptake in three grasslands using ¹⁵N labeled NO₃ and NH₄. Specifically, we tested if N-uptake patterns change across communities depending on species composition and diversity or if N-uptake patterns are persistent across different community types. Plant species in the investigated grasslands showed little chemical, spatial and temporal differences with respect to N-uptake. However, depending on quantitative N-uptake and N-use strategy, the different plant species separated into complementary functional groups. Across different grasslands, species composition of a community showed no effect on the plants' N-uptake suggesting that plants within functional groups are consistent in their N-use strategy across different communities. Consequently, the loss of a functional group from an ecosystem cannot be compensated by species from other functional groups.

13.13.5. The process of plant community degradation of two communities-*Leymus chinensis* and *Stipa grandis* grassland, Inner Mongolia, China

H. Tang;
College of Resources Science and Technology, Beijing, China.

Two indicators of plant community degradation are reduction of vegetation cover and decline in land productivity. Intensive human activities, especially overgrazing has been promoting a progressive degradation in grassland. It is necessary work to figure out the process of degradation in order to manage the grassland ecosystem well and efficiently. Three sites were chosen to demonstrate community degradation over the past decades in Xilinguole League, Inner Mongolia. Plant community species composition, aboveground biomass and soil properties were

collected at each site. Our results suggested that heavy grazing is one of the major cause of degradation. Plant species number in a site decreased from 55 in 1997 to 38 in 2001 and to 14 in 2004. Plant aboveground biomass declined from 4402.5 kg/hm² in 1979 to 708.3 kg/hm² in 2004 in one site, from 3409.9 kg/hm² in 1995 to 1958.6 kg/hm² in 2004 in another site. Up to 2005, about 90% of the 4 million km² natural grassland in China was degraded at different levels. Our research provided solid data and new understanding of plant communities degradation of grassland in north China.

13.13.6. Grassland diversity conservation in the central Apennines (Italy)

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The abandon of rural activities in the mountainous areas may increase environmental hazard and reduce biodiversity. The objective of this study was to identify and characterise the current livestock management and the corresponding grassland biodiversity in a pastoral area where traditional livestock activities are still practised. The area includes three pSIC with habitat *6210, which is an important orchid site. Botanical, agronomic and livestock management data were processed with a GIS. Areas with similar grazing value and management were identified and clustered. *Xerobromion* grasslands showed the highest stocking rate, while in some *mesobromion* grasslands it was too low to preserve them from degradation. The uneven spatial distribution of the stocking rates was recognised to be one of the most important factor for grassland degradation and shrub re-establishment. To maintain the current grassland diversity, it would be desirable to exploit the grazing behaviour of different animal species also introducing some devices (e.g. water points) to redistribute the stocking rate according to the carrying capacity of the different areas.

13.13.7. Biodiversity of semi-natural grasslands under human pressure in Central Europe

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Despite the semi-natural grasslands belong to the most species-rich ecosystems, their area and habitat quality are decreasing due to changing land use, abandonment, fragmentation and isolation. The decline of plant species richness, changes in composition and disturbance of speciation processes are results. Decrease of grassland area is documented on national, regional and local levels using statistical and remote sensing data from several countries. Changes in community structure of mountain and wet meadows in Slovakia during last 30 years are evaluated on the basis of long-term research data. Using species composition data set in different phases of managed and abandoned grasslands in Slovenia, we quantified and correlated patterns of change in frequency, and species richness. We also made models of possible scenarios particularly for speciation processes of three taxa. Threat status of grassland species and communities is evaluated using analysis of red lists and books for Slovakia, Slovenia, Czech Republic, and Hungary. The main reasons of grassland changes and possibilities for situation improvement are discussed in broader context of Europe.

13.14.1. Ethnobotanical studies of afghan refugees camps in Pakistan

S. S. Gilani, Z. K. Shinwari;

Kohat University of Science and Technology, Kohat, Pakistan.

A survey was conducted in 16 Afghan refugees' camps in NWFP province of Pakistan, to document the local medicinal uses, socio-economic surveys of camps, market surveys of plants and impact of the existence of Afghan refugees on the local flora. The local Afghan refugees used 60 plant species for curing various diseases. Inhabitants of camps consumed fuelwood, annually, 5,099,578.02 Kg in summer, and 6,871,771.8 kg in winter, respectively. They brought fuelwood either from the market (45%) or collected (55%) from the near mountains. Timber smuggling of *Pinus roxburghii* and *Cedrus deodara* from Afghanistan to Pakistan was a common practice. Due to heavy pressure on forests in the surrounding areas of camps, for fuel-wood, dominance of local

flora was decreased. Afghan refugees used 25 fodder species both cultivated and wild. The grazing pressure on the surrounding area was high due to their larger herds of cattle. The study in Refugee camps in Kohat, Hangu and Karak districts of NWFP also estimated that *Nannorrhops ritchiana* was harvested, around 2000 tons, annually.

13.14.2. Impact of two recent wars on biodiversity of Afghanistan

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¹Kohat University of Science and Technology, Pakistan, Pakistan, ²University of Karachi, Pakistan, Pakistan.

Forests are important natural resources of energy and construction material. Trees like pistachio and almond provide nuts that supplement diet and generate income. Due to lack of communication, many of the earlier remedies for various diseases survived only orally from generation to generation and are practiced in remote areas. Scientific knowledge on biodiversity in the tribal areas of Afghanistan and Pakistan is very scanty. In regions where monsoon has a considerable effect, the tree-line can easily be drawn in different regions. Soviet occupation of Afghanistan and civil wars had repercussions for tribal belt between Afghanistan & Pakistan forcing the people to shift to Pakistan. They indiscriminately harvested the plants and destroyed the natural reserves. Drug/timber smugglers of the area are very influential and offer attractive wages to wood cutters leading to the destruction of biodiversity and the introduction of poppy. Positive sources offer little to the locals. The focus should be to make inventories, reconstruct herbaria, transfer knowledge to Afghans and build their capacity to record it.

13.14.3. Phytogeography of flowering plants of afghanistan

M. Qaiser;

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Three phytogeographical regions of Afghanistan are recognized. Most of the country belongs to Irano-Turanian region. The bulk of flora (>56.2%) is made up of this element. The southern part belongs to Saharo-Sindian region. The more moist eastern part with summer rainfall is tentatively placed in Sino-Japanese region. This part may be treated as transitional zone between Irano-Turanian and Sino-Japanese region as the climatic data and distribution pattern do not support its inclusion in Sino-Japanese region. Irano-Turanian region is a center of endemism and diversity for families like Labiatae, Compositae and Leguminosae. Genera like *Astragalus*, *Eremostachys*, *Cousinia*, *Dionysia* etc. have center of diversity in Afghanistan and radiating from there are number of species. *Astragalus* has maximum number of endemic species (c. 168 spp.), followed by *Cousinia* (c. 83 spp.). The heavy bombing has created a devastating effect on the natural environment. This may have lead to the extinction of several endemics and also being mutagenic in nature, may also be responsible for creating new taxa. An objective re-evaluation of the endemic taxa therefore seems desirable.

13.14.4. Ecology of alpine plateau pastures at borderlands of Afghanistan

A. A. Dasti, S. M. Wazir;

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In species poor alpine pastures located between Pakistan and Afghanistan, we evaluated evidence for plant species associations with physical habitat variables. A total of 42 species were included in the analysis. Data on the composition of plant communities were gathered along a 72Km long transect between 2650 and 3400 m a.s.l. Soil samples were collected to document edaphic conditions of the area. Spearman's Rank Correlation Coefficient was used to detect relationship between environmental factor and species distribution. In Detrended Correspondence Analysis and cluster analysis, based on species presence/absence data, samples (stands) segregated into five groups. These corresponded closely with the physiographically defined habit types assigned in the field. The vegetation patterns revealed are discussed in relation to geomorphological factors and problems of plant assemblage in vegetation having widely scattered plants. The results suggested that the distribution and abundances of many species are influenced by local heterogeneity in physical habitat variables. However, there were several species that showed no habitat association.

13.14.5. Invasive Weeds: A major threat to biodiversity in Afghanistan**K. B. Marwat;**

NWFP Agricultural University Peshawar, Pakistan, Pakistan.

Afghanistan has a total area of 647,000 sq. km lying between 29° 38' N and 62° 72' E with elevation ranging from 258 m to 7485 m without any direct access to sea. With Soviet invasion in 1979 till these days, this area has been under tremendous disturbance; these include natural calamities, like drought etc. and simulated ones, like war and bombing by various forces from within and outside Afghanistan. Drought alone has damaged 30 - 35% Orchards in N-E parts of Afghanistan. These disturbances have resulted in change of agricultural practices and forest management as well as changes in micro-climatic level which in turn has paved way for invasion of alien weeds as well as evolution of new ecotypes of the native weeds, which have become invasive now. Thus during this period of about 25 years, Afghanistan's natural and Agro-biodiversity was severely affected by invasive weeds, mainly woody and perennial ones. The present paper discusses the threat posed by such weeds, their status as Alien or local invasive and possible management to conserve the biodiversity from the future degradation.

13.14.6. Ethnobotany of Pak-Afghan Tribal Areas**A. Rashid;**

University of Peshawar, Peshawar, Pakistan.

Two hundred and seventy-nine species belonging to eighty-one families were recorded during this study from Pak-Afghan Tribal Areas. The inhabitants of this region remained mainly dependent upon plant natural resources due to socio-political and economic reasons. Eighty-five species have been directly utilized for various purposes. The region exhibits a unique Biodiversity with rich medicinal plant wealth. Some of the interesting Reptonia elements are met with in this zone. Biotic interference with indiscriminate exploitation of natural resources, habitat loss, induction of unfriendly species, unstable socio-economic conditions of the inhabitants, huge influx of Afghan refugees in the territory of Pakistan during the last 30 years and recent coalition bombardment have differentially affected the species distribution and the abundance categories. This mass scale destruction of vegetation has caused even drying of the natural springs at places. The entire scenario has resulted in the great loss to the plant natural resources of the region in general and ethno-medicinal wealth in particular.

13.15.1. What do children bring to plants? What do children get from plants?**M. J. Reiss,** S. Dale Tunnicliffe;

Institute of Education, University of London, London, United Kingdom.

What sense do children make for themselves when they look at plants? We carried out two studies. One study was carried out in schools where six botanical specimens were shown individually to pupils aged 4, 8, 11 and 14 years. The children were asked a series of questions about each specimen. The results showed that the children used anatomical features in recognising and then naming the specimens and that home was far more important than was school in their recollections of where they learnt the information they told us. In the second study conversations of primary age children (5-11 years) and the adults with them were recorded in a botanical garden as they walked around the exhibits. Again, children talked about easily observable features such as colour, shape and smell and only attended to less obvious features when cued. Older children made more comments focused on plants while pupils in groups without an adult asked fewer questions of each other and commented more about the human use of plants. We interpret our results within a framework that emphasises the importance of building on pre-existing conceptualisations.

13.15.2. enhancing botanical literacy in the public school classroom through the national science foundation teaching fellowship program at the University of Maine**S. Brown¹,** M. Doing², S. Cromley³;¹George B. Weatherbee School, Hampden, ME, United States, ²Dr.Lewis S. Libby School, Milford, ME, United States, ³University of Maine, Orono, ME, United States.

In an ever-changing environment where plants are under constant pressure from development and habitat fragmentation, botanical literacy is critical to plant conservation. The National Science Foundation Teaching Fellowship Program (NSF GK-12) offers students and teachers in the public schools opportunities for scientific enrichment through the expertise of undergraduate and graduate students studying science, math, and engineering. A major goal of this program is to enhance public school education by providing equipment, hands-on activities, field trips, and exceptional role modeling for students. Several University of Maine participants have developed plant-related units for use in K-12 classrooms. These include germination experiments, flower dissections, plant pressing and identification, problems associated with invasive species, plant adaptations, and creating and using dichotomous keys. We provide a summary of unit objectives and various associated activities.

13.15.3. Building botanical literacy in South African schools**M. C. Doidge;**

University of Witwatersrand, Johannesburg, South Africa.

Botany forms a relatively unpopular component of the high school biology curriculum amongst both teachers and pupils in South Africa. The emphasis on plant anatomy and physiology and on the life cycles and characteristics of different phyla no longer generates much interest in botany amongst South African students and this is reflected in the decline in enrolment in botany in universities. However, a new outcomes-based curriculum is in the process of being introduced into South African schools. This new curriculum promotes the development of skills, values and attitudes in addition to the construction of knowledge. Thus although the amount of botanical content has been reduced considerably, botanical literacy may in fact be encouraged. This paper describes the changes in the new curriculum with regards to botanical education over the first twelve years of schooling. It then reports on a survey amongst botany tertiary educators on the impact of the new curriculum on general botanical literacy as well as its adequacy in preparing pupils for tertiary education or for jobs in the field of botany.

13.15.4. Trends in botany education in U.S. Colleges and Universities**M. D. Sundberg;**

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During the past 30 years several trends in U. S. botanical higher education are clear. On the negative side, the number of botany and/or plant science departments has declined at 0.65 per year and there has been a drastic decline in traditional course offerings, particularly in taxonomy and floristics. This decline is most evident at smaller institutions but even some of the largest universities with traditionally strong botany departments are now experiencing erosion of botanical course diversity. On the positive side, the number of botany graduate students has remained essentially constant and the number of botany faculty (now frequently in biology departments) has increased 0.26/year. Much of this increase focuses on cellular and molecular plant biology, particularly at research universities. However, several examples will be presented where departments maintained or developed new strength through focusing on well-defined missions and/or establishing collaborations with gardens or museums.

13.15.5. Teaching systematic botany at German Universities - Facts, challenges and opportunities.**M. Weigend;**

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In the past decades systematic botany at German universities has continuously declined, the number of classes, lectures and practical courses has decreased. Courses such as "plant identification" are no longer part of a biologist's university education and many important fields (e.g., phycology, mycology) are no longer covered in the curricula. At the same time systematic botany is currently more rapidly developing than ever before and new aspects have to be continuously incorporated into fewer and fewer hours of class work. There are two basic approaches towards improving the quality of botanical education at university: 1) Improving and stream-lining the existing courses (e.g., improving teaching aids, internet resources), co-ordinating teaching with other botany departments (e.g., student exchange)

and the teaching of general techniques (e.g., molecular techniques) with other institutes at the same universities. 2) Systematic botany has to improve its image and centre-stage its role in society. This can i.A. be done by outreach programmes and press releases. Systematic botany has to become visible to become respected.

13.15.6. Activities and ambassadors: Creating educational programs from existing plant collections

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Existing plant collections may be in the form of gardens, arboreta, herbaria or University grounds. In many cases, botanists understand their value to their courses and research but are not readily prepared to reach out to other groups, especially with the limited time available to them. Unfortunately the value of Natural History Collections, living or dried, is not well appreciated outside of the user community. Outreach that combines a collection's individual strengths with the interests or needs of target audiences can give a greatly needed. With the right preparation, professors or students train volunteers and others to give tours or programs that they create. The keys to success are 1) simplifying the program development to be quick and easy, 2) picking the ambassador plants to carry your message, 3) choosing activities that play to your target audience and 4) knowing the market realities of your target audience. Case study data and real world experience show support for the "Activities and Ambassadors" approach but also lessons for whatever outreach method is employed.

13.15.7. A research-based model for writing interpretive botanical trail signs

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Outdoor interpretive signage systems can provide reliable, trailside, plant science information every day of the year, without the need for added site personnel to serve as guides or the continuing expense and litter potential of printed trail guides. When we discovered an exemplary outdoor trail signage system at Mississippi State University's Crosby Arboretum, we conducted an in-depth content analysis of all 158 interpretive signs, in order to: (1) uncover what textual and design elements made the Crosby Arboretum signage system's trail signs seem more interesting and readable to us than any we had seen previously worldwide; (2) help plant science instructors choose field trip sites wisely by assessing the *opportunity to learn* from interpretive science signage found along established outdoor trails; and (3) assist those who wish to design a public, interpretive plant science signage system for a nature trail at a school, nature park, arboretum, or botanic garden. Our findings led us to develop our research-based quantitative and qualitative model for writing interpretive botanical trail signs that is currently being applied at several sites across the US.

14.1.1. Plant food allergens: Structures, characteristics and relationships

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A wide range of proteins present in plant-derived foods have been reported to result in allergenic symptoms following prior sensitisation via the GI tract or to related proteins present in pollen. Although they occur in a diverse range of foods the vast majority of the proteins that sensitise via ingestion fall into only two superfamilies of plant proteins, called the prolamin superfamily and the cupins. We will compare the structures and evolutionary relationships of both allergenic and non-allergenic members of these superfamilies. We will also speculate on the relationship between their allergenic properties and biological activities and discuss how our knowledge of these relationships can be used to develop risk assessment procedures for novel and GM foods. We will also speculate on the possibility of using GM or other new technologies to reduce the contents or eliminate allergenic proteins from foods.

14.1.2. A structural classification of pollen allergens

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Atopic individuals can be sensitized by pollen from a great number of conifer species and monocotyledonous as well as dicotyledonous species of flowering plants. Despite the diversity of sensitizing pollen sources, most pollen allergens can be classified into a small number of protein families. The Allergome database contains 129 pollen allergens with complete or nearly complete sequences. 125 of these sequences can be grouped into 24 known protein families. The ten most abundant families contain 80% of all allergens. The most widely distributed pollen allergen families are the profilins and calcium-binding proteins containing EF hand domains. Other nearly ubiquitous families are the Ole e 1 homologues and the polygalacturonases. A large number of grass pollen allergens belongs to the expansin family. Additional allergen families with a limited species distribution include pectate lyases (Cupressaceae and Asteraceae), ribonucleases (Poaceae) PR-10 pathogenesis-related proteins (Fagales), thaumatin-like proteins (Cupressaceae) and flavoproteins (Poaceae). This work was supported by grant SFB-F018-02 from the Austrian Science Fund.

14.1.3. Structure and allergenicity of food allergens from the PR-5 family

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Thaumatin-like proteins (TLPs) belonging to the PR-5 family of pathogenesis related proteins have been identified as allergens from plant foods and pollen. We recently succeeded in crystallizing two members of this allergen family: the TLP isolated from ripe cherries, Pru av 2, a 23 kDa protein was crystallized in space group P2₁ with unit cell parameters a=44.8Å, b=41.8Å, c=59.6Å, beta=106.8°, and the major kiwi allergen, Act c 2, a 22 kDa protein was crystallized in space group C2 with cell dimensions a=71.6Å, b=48.5Å, c=50.9Å, beta=100.56°. Both crystal forms diffracted to atomic resolution at the synchrotron. The structures were solved by molecular replacement and the model building and refinement is in progress. In this paper the allergen structures will be compared with the known structures of other TLP proteins such as zeamatin, thaumatin, osmotin and PR-5d, and the correlation between structure and cross-reactivity will be discussed. Acknowledgements: This work was supported by the Austrian Science Fonds (FWF) projects F01805 and F01802.

14.1.4. Localisation of Bet v 1 and Bet v 7 in the anthers of birch, hazel, and rye grass

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Pollen of birch is one of the main causes of allergic reactions in Europe. In recent studies evidence is supplied that allergenic activity in the micronic aerosol may play an important role in causing allergic reactions. In the anthers of flowering plants tiny (< 4 µm) granules can occur on the locule walls. These granules are called orbicules or Ubisch bodies. The hypothesis of the potential role they may play as vector of allergens is tested by investigating the localisation of the birch allergens *Bet v 1* and *Bet v 7* across the birch anther using immunogold electron microscopy. The localisation of cross-reactive homologs of these allergens is studied in the anthers of hazel and rye grass. The results indicate that orbicules and pollen of birch, hazel and rye grass might act as effective vectors for *Bet v 7*. In birch and hazel orbicules only minor labelling occurred for *Bet v 1*. The immunologic consequences of these localisation studies are discussed.

14.1.5. Art v 1, a defensin-like allergen from *Artemisia vulgaris* pollen

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¹Department of Molecular Biology, University of Salzburg, Salzburg, Austria, ²Department of Pathophysiology, Medical University of Vienna, Vienna, Austria, ³Department of Chemistry, Universitaet für Bodenkultur, Vienna, Austria.

In late summer, pollen grains from Asteraceae weeds are a major source of allergens worldwide. Ninety-five percent of patients allergic to mugwort (*Artemisia vulgaris*) are sensitized to the major allergen, Art v 1. The N-terminal domain of Art v 1 shows a cysteine stabilized (alpha/beta) motif typical for plant defensins. The C-terminal cysteine-rich region consists of several (Ser/Ala)(Pro)2-4 repeats and is predicted to adopt a left-helical conformation similar to collagen. Proline residues are hydroxylated with a frequency of 78-80% and a new type of plant O-glycans are attached during post-translational modification. Evaluation of the antibody binding properties of natural and recombinant Art v 1 produced in *E. coli* demonstrated the involvement of the defensin folding and post-translational modifications in the formation of epitopes recognized by IgE antibodies from allergic patients. The T cell response of Art v 1 is characterized by one strong immunodominant epitope and is associated with the expression of HLA-DRB1*01. This work was supported by grant FSP S88 (S8802, S8803, S8808) from the FWF, Austria.

14.1.6. Expression of the latex allergen Hev b 2 in bacteria and tobacco plants

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The use of purified allergens allows a molecule-based diagnosis and therapy of allergies instead of the currently used allergen source-based assays, e.g. skin prick tests with pollen extracts. A low-costs production of high amounts of recombinant allergens can be achieved by molecular farming strategies; an ideal prerequisite for a wide use of recombinant allergens by medical practitioners in future. Additional benefits of the plant-based production of allergens are the specific post-translational modifications and the correct protein folding that are unlikely in non-plant expression systems (bacteria, yeast, mammalian cells). The latex allergen, Hev b 2, is a glycosylated β -1,3 glucanase containing vacuolar targeting sequences (VTS) and so far, no recombinant Hev b 2 is available. The Hev b 2 sequence was cloned into a bacterial expression vector including a Strep-tag but without the VTS and in a plant expression vector including a His-tag and the VTS. Both expression systems were compared in respect to their ability to produce a recombinant Hev b 2 protein that is recognised by IgEs from latex allergy patients.

14.1.7. Characterisation of different allergens in Rosaceous fruits

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Regular consumption of fruits and vegetables is generally encouraged in the European Union to enhance health and prevent cancer and cardiovascular diseases. However, certain fruits or vegetables may pose a risk to atopic individuals since up to 70% of birch pollen allergic persons react with proteins homologous to pollen allergens that are expressed in Rosaceous fruits. In the frame of the present work, we focus on the characterisation of different allergens also known as pathogenesis-related proteins, e.g. PR-5, PR-10, PR-14, in fruits, which are consumed in high amounts across Europe, such as apple, pear, peach, apricot, strawberry and raspberry. To this purpose different diagnostic and analytical techniques have been employed, e.g. ELISA, Real Time PCR, ITP (Immuno Tissue Printing) and Western Blot. This methodology has allowed a first insight into localisation and cultivation- and storage method-dependent expression levels of allergens within fruits. Attempts are underway to understand the biological function of these proteins. Financed by contract SAFE, QLK1-CT-2000-01394 and Hochschuljubiläumsstiftung der Stadt Wien, H-998/2004

14.2.1. Seed size and the new fusion between ecology and evolution

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Seed size is a trade-off between maximizing the numbers of offspring produced and maximizing each offspring's chance of survival. The resulting compromise has been struck at seed sizes that vary enormously across species. A larger body of data has accumulated worldwide for seed size than for any other species

trait. Therefore seed size research is a testing ground for a new fusion between ecology and evolution, where we seek to explain the spread of a functional trait across the full range of present-day species worldwide, and at the same time we narrate the whole evolutionary history of a trait. The most consistent theme emerging from this scale-shift has been the strong association between evolutionary divergence in size of the vegetative plant at adulthood, and evolutionary divergence in seed size. How can this theme be harmonized with the strong experimental evidence indicating the benefit of larger seed size is improved seedling survival per week under various hazards? New developments in life-history theory are needed.

14.2.2. Evaluation of the tradeoffs between light capture, hydraulics and biomechanics with a 3-dimensional architecture model, Y-plant.

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The efficiency of light capture of a crown can be enhanced either by changes in leaf angles or by the repositioning of leaves in space so that self-shading is minimized. Studies with shrub *Psychotria* species native to a Panamanian tropical forest understory revealed that leaf angles were near optimum for maximizing light capture. However light capture efficiencies were found to be surprisingly low because of significant self-shading. Simulations with the 3-D functional-structural model Y-plant were used to explore the potential constraints on minimizing self-shading. Simulations in which biomass allocation between internodes and leaves was varied revealed that increasing internode length at the expense of leaf area enhanced light capture efficiencies and whole plant carbon gain. However, the biomass costs of maintaining sufficient biomechanical support and hydraulic conductivity are suggested to be constraints on the realized light capture efficiencies of these species.

14.2.3. Hydraulic architecture and transport efficiency

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Plant hydrovascular systems exhibit two pervasive traits: a trunk-to-twig decline in xylem conduit diameter, and in the leaf-specific conductivity of the axis. These traits reduce the frictional resistance per vascular investment. The steeper the trunk-to-twig declines in diameter and conductivity, the fewer and wider are the conduits in trunk vs. twig, and the greater the transport efficiency. Greater efficiency, however, causes top-heavy vasculature and mechanical vulnerability. This consequence limits maximum efficiencies to growth forms like vines where conduits are not mechanically significant. The tendency for trees to follow Da Vinci's area-preserving branching rule may be a compromise between hydraulic efficiency and mechanical stability. Hydraulic efficiency is also limited by maximum conduit diameter. Diameters may be limited by both mechanical considerations and by susceptibility to cavitation, with mechanisms differing between conifer tracheids and angiosperm vessels.

14.2.4. How do palmate and pinnate leaf venation systems differ in function? Divergence in the impacts of damage on hydraulics and gas exchange

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Leaves of dicotyledons vary tremendously in their venation architecture, but little is known of the potential functional consequences. One striking dichotomy is between palmate and pinnate arrangement of major veins. We applied cutting treatments to the veins of leaves on naturally occurring trees of temperate woody angiosperms, for species with pinnately- or palmately-veined leaves. Once the leaves had healed the wounds, we measured performance, relative to control leaves, in leaf hydraulic conductance, in stomatal conductance and in parameters of chlorophyll fluorescence, as an index of photosynthetic function. Pinnately-veined leaves were inherently more sensitive to damage: severing the primary vein dramatically reduced leaf function. By contrast, in palmately-veined leaves, severing the central primary

vein reduced function much less strongly, especially further from the cut, with the surrounding primary veins compensating for the loss of water supply capacity. Functional differences among leaves of contrasting vascular design have implications for leaf evolution, ecology and biogeography.

14.2.5. Hydraulics and mechanics of growth form evolution

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Different plant growth forms possess different combinations of hydraulic and mechanical function. Trees, shrubs, herbs, vines and lianas can show very different functional strategies. Plant size, height and mass are parameters traditionally investigated for surveying trends in plant structural diversity but differences in plant growth form - how the plant is positioned and whether it mechanically supports itself or not - are also important. So, how is growth form diversity best understood? Combinations of functional attributes such as hydraulic and mechanical architectures can play a prominent role in developing appropriate innovations for diverse and extreme ranges of growth forms. It is of extreme interest to know how such complex functional traits appear and become potential innovations for further morphological radiations. The bifacial vascular cambium is an important innovation permitting a wide range of hydraulic and mechanical functioning. One of the most important attributes ensuring long-term survival of lignophytes was the morphological flexibility ensured by cambial development and the potential for varying growth form and body size.

14.2.6. Alternative designs and the evolution of functional diversity in tree seedlings.

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High levels of quantitative variation in functional traits exist among plant species, both within sites and among sites. This trait diversity affects species distributions as well as ecosystem processes. Trait variation among sites often can be explained by species adaption to major environmental differences, but explaining the large variation observed within sites poses more of a challenge. We present the evolution of alternative functional designs of approximately equal fitness in the same environment as an explanation. We use a genetic algorithm and a realistic model of tree seedling growth and survival to demonstrate how the numerous tradeoffs associated with the multifunctional nature of tree seedling designs result in the evolution of many alternative, but functionally equivalent designs. These alternative designs show convergence in terms of growth rate and survival in a given environment while retaining high diversity in individual traits. Changes in environment shift the range of potential values for many traits but do not preclude the evolution of diverse alternative designs.

14.2.7. Climate and land plant architecture - general patterns and individual solutions

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For all land plants, climate is an important environmental parameter influencing not only the distribution and physiology but also the architecture of land plants. In particular it is well known that the distribution of life forms as well as the occurrence of wood anatomical and of leaf morphological characters is largely controlled by climate although the functional relationship between climate and land plant architecture is still poorly understood. Here we test to what extent certain relationships between land plant architecture and climate are controlled by physico-chemical principles and/or by the systematics and evolutionary history of the plants. The result has significant implications for palaeoclimatology since the Recent relationship between features of land plant architecture and climate is frequently used to reconstruct the climate of the geological past.

14.3.1. Structure, function and biosynthesis of arabinogalactan-proteins (AGPs)

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Arabinogalactan-proteins (AGPs) belong to the Pro/Hyp-rich family of complex glycoproteins. In *Arabidopsis*, they include classical AGPs, AG-peptides, basic AGPs and fasciclin-like AGPs (FLA) (Johnson et al, Plant Physiol. 133:1911). We are focussing on FLAs because they include putative cell adhesion domains. The identification of a salt overly sensitive (*sos5*) mis-sense mutant in the H2 region of *AtFLA4* (Shi et al, Plant Cell 15: 19) indicates that these domains are important for FLA function. We are now comparing *fla1* and *fla4* (*sos5*) mutants. We are also focussing on the (1-3) galactosyltransferases thought to be involved in the assembly of the AG chains in AGPs. These Golgi-located enzymes belonging to family GT31 in the CAZY database (<http://www.afmb.cnrs-mrs.fr/~pedro/CAZY/db.html>). We are studying the expression patterns, sub-cellular locations and enzymatic activity of 5 distinct members of this family. This work is supported by an ARC DP grant (DP0343454).

14.3.2. Lysine-rich arabinogalactan-proteins (AGPs): structure, localization and function

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Lys-rich AGPs comprise a subfamily of hyperglycosylated, hydroxyproline-rich AGPs which decorate plant cell surfaces and include LeAGP-1 in tomato and AtAGP 17, 18 and 19 in *Arabidopsis*. Overexpression of LeAGP-1 results in tomato plants that were significantly shorter, were highly branched, produced less fruit, and produced seeds that were significantly smaller than normal seeds. The phenotype of these transgenic plants is similar to that of cytokinin-overproducing plants, and LeAGP-1 mRNA expression is up-regulated by cytokinins. We speculate that LeAGP-1 is an extracellular mediator of cytokinin. In *Arabidopsis*, a T-DNA knockout mutation in AtAGP19 was obtained. Compared to wt plants, the mutant had: lighter green leaves containing less chlorophyll and anthocyanins, rounder leaves with shorter petioles, shorter and thinner inflorescence stems, slower growth with delayed and reduced flowering, fewer siliques and seeds, and fewer lateral roots. Complementation with the wt AtAGP19 gene restored wt phenotypes. Consequently, AGP19 functions in plant growth and development of multiple plant organs consistent with its RNA expression pattern.

14.3.3. Control of cell elongation by xyloglucan fragment oligosaccharides

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Xyloglucans, which occur in the primary and secondary walls, are composed of 1,4-beta-glucan backbone with 1,6-alpha-xylosyl residues along the backbone. Xyloglucans cross-link adjacent cellulose microfibrils to form tethers in the walls. Xyloglucan endotransglucosylases (XETs) in the walls rejoin a xyloglucan chain to another chain by transglucosylase reaction. In the previous work we have already demonstrated that the integration of whole xyloglucan caused the suppression of cell elongation by the action of wall-bound XET, whereas that of its fragment oligosaccharide (XXXG) accelerated growth. In the present work, we examined the elongation of pea stem segments by the addition of a series of xyloglucan heptasaccharides (XXXG), 1 to 10 units of XXXG (1 to 10 kD). The results showed that the fragment oligosaccharides composed of 1 to 4 units accelerated and those composed of more than 6 units of XXXG suppressed cell elongation.

14.3.4. Di-isodityrosine in extensin analogs crosslinked in vitro

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Extensins are cell wall hydroxyproline-rich glycoproteins that form covalent networks catalyzed by extensin peroxidases. To identify the precise crosslinks involved, we designed a set of extensin analogs encoding tandem repeats of the P3 motif: Ser-(Hyp)4-Ser-Hyp-Ser-(Hyp)4-Tyr-Tyr-Tyr-Lys, including Tyr to Phe and Lys to

Leu variations. Expression of these P3 analogs in tobacco cells yielded glycoproteins with all Pro residues hydroxylated and subsequently arabinosylated and with galactosylated Ser. This was consistent with earlier analyses of P3 glycopeptides isolated from cell wall digests and the predictions of the Hyp contiguity hypothesis. The tyrosine-rich P3-analogs also contained isodityrosine, formed *in vivo*. Significantly these isodityrosine-containing analogs were further crosslinked *in vitro* by an extensin peroxidase to form the tetra-tyrosine intermolecular crosslink amino acid di-isodityrosine. This is the first identification of an intermolecular crosslink amino acid in an extensin module and corroborates earlier suggestions that di-isodityrosine represents one mechanism for crosslinking extensins *in muro*.

14.3.5. Structure and interaction of HRGPs in *Chlamydomonas reinhardtii*

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Extracellular hydroxyproline-rich glycoproteins (HRGPs) in the unicellular green alga *Chlamydomonas reinhardtii* serve two functions: they are constituents of a non-cellulosic cell wall and mediate sexual interaction of gametes of opposite mating type. The sequenced HRGPs in *Chlamydomonas* all prove to be chimeric, with globular and fibrous (shaft) domains. Shaft domains are in a PP II configuration and their amino-acid sequences fall into families of repeated motifs (e.g. P_{Sn}, PPX_n, PPSPX_n), where a given shaft often carries several discrete repeating subdomains. Both, agglutinins and cell wall proteins interact and form characteristic meshworks. The self-assembly of outer wall proteins *in vitro* was shown to produce the same lattice organization as *in vivo*. In a recent study, we have used isolated outer wall GPs and analyzed their interactions under equilibrium and real time conditions. The data obtained enable us to quantitatively describe the formation of the outer non-covalently crosslinked cell wall.

14.3.6. Mutations affecting pectin formation suppress the *Arabidopsis* cell wall formation mutant *lrx1*

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Extracellular leucine rich repeat-extensin proteins (LRX) are likely to have a regulatory role during cell wall formation. The paralogous *Arabidopsis* genes *LRX1* and *LRX2* are expressed in root hairs and *lrx1* mutants develop deformed root hairs. This mutant phenotype is enhanced in the *lrx1/lrx2* double mutant. Ultrastructural analysis of *lrx1* and *lrx1/lrx2* mutants revealed an aberrant cell wall structure. Thus, LRX1 and LRX2 synergistically interact and are important for root hair cell wall formation. A suppressor screen was performed on the *lrx1* mutant and several *rol* (repressor of *lrx1*) mutants were isolated that display wild-type root hairs and thus suppress the *lrx1* phenotype. Two alleles of *rol1* were identified and map-based cloning revealed mutations in a gene that appears to be involved in pectin biosynthesis. We are currently characterizing the cell wall sugar composition and -structure of wild-type, *lrx1*, *rol1-1/lrx1*, and *rol1-2/lrx1* plants. This analysis will possibly allow determining whether the suppressive effect in the *rol1* mutant is specific and thus LRX1 is important for the proper formation of the pectic matrix of root hairs.

14.3.7. Arabinogalactan-proteins and plant cell expansion

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Arabinogalactan-proteins (AGPs) are a complex class of plant proteoglycans that occur at plasma membranes and cell walls. AGPs consist of diverse galactan-based heteroglycans that can be associated with a diversity of core proteins. Several lines of evidence indicate that AGPs have fundamental roles in plant biology. The presentation will focus on the possible function of AGPs in plant cell expansion processes. Using a diversity of experimental approaches including Yariv-reagent inhibitors of AGP action, monoclonal antibodies to AGP glycans and genetic dissection, evidence for the involvement of AGPs in the extension of apical cells of protonemata of the model bryophyte *Physcomitrella patens* will be described. *Physcomitrella* protonemata extend by a tip-growing mechanism that is sensitive to AGP-binding Yariv reagents. Bioinformatic and biochemical

analyses of *Physcomitrella* AGPs have provided insight into the structure of bryophyte AGPs in relation to those of higher plants.

14.4.1. The evolution of character coding

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All morphological systematists from Linnaeus and Ray and onwards have been faced with the problem of describing observed variation as a recognizable suite of characters and states. While great authorities such as Linnaeus and Hennig undoubtedly encountered this problem, it was only with the introduction of numerical methods in the 1950s, however, that this subject was explicitly discussed, thus giving rise to character coding as a field. Early methods were inadequate, both for expressing information and for use in phylogenetic inference. While more recent methods have solved many of those problems, some confusion has persisted in the literature. Interest in molecular evolution at first led to reduced emphasis on any but trivial coding methods, but more complete use of molecular information, such as indels and other structural rearrangements, gene order and gene expression, seems likely to raise the same kinds of coding problems seen with morphological data.

14.4.2. Character coding for phylogenetic analyses of structural and functional genomic data

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Data bearing on the structure and function of genomes is accruing exponentially. However, development of tools to describe heredity-based hierarchical patterns and processes has lagged behind. For structure, reconstruction of organismal phylogeny can be based on 1) gene order among multiple genomes, or 2) gene presence vs. absence. Also possible 3) is optimization of genomic content (genes +/-) onto *a priori* trees to estimate ancestral gene complements at nodes. For 1), the state space provided by enormous numbers of alternative character states (synteny orders) provides a parsimony/likelihood equivalence. For 2) and 3), lengths of strings under comparison (genes) mean that Dollo parsimony coding will also be likelihood equivalent. However, just as with standard phylogenetic analyses, lateral transfer can breach an hierarchical process assumption. Phylogenetic methods are also appropriate for examining functional genomic data if such information has an underlying hereditary basis. Microarray expression profiles from different organisms can be analyzed cladistically with explanatory power unavailable from popular phenetic clustering methods.

14.4.3. Homology and the circumscription of characters

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Characters are the fundamental hypotheses on which all phylogenetic analyses are based, meaning that the decision about how observations are translated into characters is of paramount importance in systematics. Some current character definitions are inadequate because they fail to circumscribe the character clearly. Moreover, the distinction between character and character state is accepted by many but rejected by others. Important issues to consider in circumscribing characters are how organismal variation originates and whether units of variation are independent of each other. Here, the position is taken that characters correspond essentially to genetic paralogs and their states to their orthologs and this distinction should be embraced as a paradigm for all data types. This approach provides a non-arbitrary circumscription for characters. Furthermore, there are practical coding implications that follow from the way that characters are viewed and these need to be considered when studies of real organisms are undertaken.

14.4.4. A pluralistic approach as a global solution for phylogenetic analysis of complex data sets

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Positional homology assessment of nucleotides and amino acids when comparing multiple sequences is tightly linked to phylogenetic inference. Length variation among sequences being considered for phylogenetic analyses represent a special and complex case for which there is no optimal single solution.

Because indels are concentrated in distinct regions of alignments, decoupling the alignment process from the phylogenetic search allows a pluralistic approach where informed decisions can be made for each segments of the alignments containing at least one indel. Solutions for simple to highly complex alignment regions containing indels will be demonstrated using empirical results. Congruence among data partitions, efficiency of phylogenetic methods, availability of characters without indels, and the number of sequences (or taxa) part of the alignment are factors to be considered for finding optimal solutions.

14.4.5. Homoplasy is ignorance: Enlightenment through reciprocal illumination.

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The naming of characters and character states implicitly contains a *priori* homology statements. While morphological characters may be difficult to assess and code *priori*, they do lend themselves to testing via cladistic analysis and reciprocal illumination, i.e., a *posteriori*. There is no homoplasy in nature only our failure to see the difference. Consider the occurrence of perisperm in *Acorus* and *Hydatella*. What initially appears as homoplasy in a character state because it occurs twice on a cladogram may, in fact, be precisely what the cladogram indicates: two different things. In fact, development has shown this because the former is dermal in origin and the latter is subdermal in origin. Thus, by extension, in DNA (probably an acronym for Don't Need Anatomy) sequence data not all As are the same. An A can be replaced by a T in a sequence and later in time that T can be replaced by an A in exactly the same position. The A's are of course exactly the same but yet not the same A's historically. Of course this does not subject itself to reciprocal illumination in the same way that morphological, phytochemical, anatomical characters do. How ignorant are we?

14.4.6. Character-based phylogenetic Linnaean classification: taxa should be both ranked and monophyletic

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The modern approach to classification relies on character-based phylogenetic patterns. Three views have emerged about the relationship between classification and phylogeny reconstruction. The first, exemplified by the PhyloCode, is that ranked classifications should be abandoned in favor of naming clades. The second view is that ranks should be maintained and that this logically requires the acceptance of paraphyletic taxa, which is the result of inappropriate interpretation of cladograms. We support the third view, that ranks should be maintained, but that taxa above the level of species should be circumscribed as putative monophyletic groups, underscoring the fact that cladistic principles and ranked taxonomy are compatible and complementary. Coupling Linnaean taxonomy with the criterion of monophyly facilitates viewing classifications as explicit, testable hypotheses of relationship. Minor changes to the ICBN can address concerns about the proliferation of monotypic taxa that could result from strict application of this idea. Such classifications do not ignore the "modification" in "descent with modification."

14.4.7. Ragged ends and gene duplications: new strategies for old problems

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Two topics will be discussed: (1) Regions of poor alignment of amino acid or DNA sequences are typically excluded from phylogenetic analyses, but regions of ragged alignment adjacent to well-aligned blocks may provide especially informative phylogenetic signal. (2) Phylogenetic analyses of duplicated genes are increasingly used to infer organismal phylogeny. The method of Double Data Entry (DDE) can be used to analyze paralogs resulting from a single duplication event. DDE combines information from the two paralogs to infer an organismal phylogeny, as does Uninode coding. DDE avoids a potential artifact arising from the prior calculation of the states at the uninode, which cannot change during the subsequent phylogenetic analysis. DDE is appropriate for parsimony as well as for other methods, such as ML or distance methods. Standard phylogenetic software does not require modification for use with DDE.

14.5.1. Evolution of cauliflory in Bignoniaceae: systematics, development and ecology

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The role of phylogenetic constraint, preadaptation and/or adaptation in the evolution of cauliflory is explored for this common but poorly understood inflorescence architecture of the tropical rainforest understory. This disassociation of reproductive from vegetative growth has stimulated a range of hypotheses relating to flower/fruit characters, tree architecture, phenology, and visual vs. olfactory attractants. Although much speculation has been generated over cauliflory, little experimental evidence has been produced, and none in a phylogenetic context. Using a comparative phylogenetic approach we examine the evolution of cauliflory within Bignoniaceae. These traits are studied across lineages, as well as compare sister taxa that are not cauliflorous. Species richness is examined as a means to explore the possibility of cauliflory as a key innovation. By comparing these patterns within and across lineages, a better understanding can be achieved of both the phylogenetic, developmental, and ecological constraints on the evolution of the cauliflorous syndrome.

14.5.2. Phylogenetical relationship of the Pithecocteninae (Bignoniaceae: Bignoniaceae) subtribe on base of morphological characters

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A phylogenetic hypothesis of subtribe Pithecocteniinae (Bignoniaceae, Bignoniaceae) is presented, derived from the analysis based on a parsimony model. The ingroup of the surveyed taxon included 33 species (out of 42 in total) of the genera *Amphilophium*, *Distictella*, *Distictis*, *Glaziovia*, *Haplolophium*, and *Pithecoctenium*, while the outgroup considered one species each of *Mansoa* and *Pyrostegia*. Forty one characters, comprising features of pubescence, gland types, floral morphology, pollen exine, and seed wing anatomy, were considered in the analysis. The resultant consensus tree of the study (out of 606 equally parsimonious trees) clearly sustain just three distinct monophyletic groups, strongly supported by statistical values of Jackknife and decay index, suggesting the recognition of only three genera: *Amphilophium* (including *Haplolophium*, and *Glaziovia*), *Distictis* (including *Distictella*), and *Pithecoctenium*. Thus a new generic classification within the subtribe is proposed.

14.5.3. Phylogeny and biogeography of the genus *Incarvillea* (Bignoniaceae)

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Incarvillea is a herbaceous and temperate member of Bignoniaceae, previously divided into four subgenera, *Niedzwedzkia*, *Amphicome*, *Incarvillea*, and *Pteroscleris*. *Niedzwedzkia* and *Amphicome* have in the past been treated as independent genera, and different relationships have been proposed for the four subgenera. We studied phylogeny of the genus based on the data from cytology, pollen morphology, DNA sequences, and analyzed biogeography of the genus using DIVA (Dispersal-Vicariance Analysis) based on the combined tree resulted by the ITS and trnL-F sequence data analysis. The results indicated that all species studied are very similar in pollen morphology, but differ in karyomorphology. Maximum parsimony analysis using ITS and trnL-F sequences resulted in similar trees and showed that the genus is monophyletic, as the interspecific similarity in karyomorphology and pollen characters implied. Biogeographic history as an explanation for the results is also explored.

14.5.4. Development and Function of Included Phloem in Woody Lianas of the Bignoniaceae

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Lianas in the subfamily bignoniaceae exhibit atypical secondary growth by developing included and internal phloem. Development of included phloem first occurs in the "corners" of the stem and is correlated with the phyllotatic pattern and vascularization. Included

phloem occurs when segments of cambium become unidirectional and produce secondary phloem but little, if any, secondary xylem. The activity of the unidirectional segments can be reversed by auxin and gibberellin. Multiseriate rays border the phloem furrow and extend the length of the internodes allowing the included phloem to slide by the stationary secondary xylem. Rays also may serve as physiological barriers to growth regulating substances that influence differentiation of cambial derivatives. Selective defoliation experiments suggest these substances are coming from the leaves. In addition, critical experiments have shown that a possible function of included phloem is allowing the liana to twist and bend without separating the vascular connection between stems and roots. When complete girdling occurs, included phloem prevents death by regenerating a new cambium and periderm.

14.5.5. Radiation and risk. Assessing threats to trees endemic to Madagascar.

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Madagascar's flora is among the most species-rich on earth. Of the roughly 12,000 known plant species in Madagascar, however, only 1.6% is found in IUCN's Red List of Threatened Species. We assign preliminary IUCN categories to the species of a comparatively well-known species-rich tribe, Coleeae (family Bignoniaceae), endemic to Madagascar. Since the IUCN Red List Categories and Criteria 3.1 discourages the use of "data deficient" classification of species, we develop a novel method for differentiating between species that are truly range-limited versus poorly-sampled, thereby "rescuing" some of the data-deficient species for status assessment, in a method that can readily be applied to other taxa and regions. Roughly 51% of the species of Coleeae are threatened with extinction, three species are presumed extinct, and an additional 11 have not been seen in decades. Due to their recent discovery, 15 species must remain in the data-deficient category until more sampling has been conducted. If Coleeae is representative of the Malagasy flora, or at least other endemic radiated plant groups, then species loss in Madagascar may be even more extreme than we realize.

14.5.6. Evolution of mammal dispersed fruits: Morphology and Anatomy of fruits in Crescentieae (Bignoniaceae)

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Fruit morphology has had great taxonomic importance in Bignoniaceae and has been used traditionally to distinguish tribes in the family. These tribes are monophyletic, with two exceptions: Tecomeae, now recognized as a paraphyletic group with plesiomorphic fruit morphology, and Crescentieae, traditionally characterized by indehiscent fruits and with members characterized by a disjunct distribution. However, Gentry suggested that indehiscent-fruited Bignoniaceae represented two lines of descent. DNA studies have shown that the historic delimitation of this tribe is incorrect and it has been split into tribes Crescentieae (Neotropics) and Coleeae (Madagascar), with *Kigelia* (Central Africa) not belonging to either. This study focuses on fruit evolution in Crescentieae. Comparative morphology and anatomy of fruits from four genera, *Tabebuia*, *Crescentia*, *Amphitecna*, and *Parmentiera* show the structural changes that have taken place in the transition from a typical dehiscent wind-dispersed Bignoniaceae fruit to an indehiscent, fleshy fruit suggested to be mammal dispersed. These changes are interpreted in light of the phylogeny of Bignoniaceae.

14.6.1. Radiations, extinctions, and evolutionary dead ends in the Amaranthaceae/Chenopodiaceae alliance

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Molecular data show a rapid formation of five major lineages within the Amaranthaceae/Chenopodiaceae alliance: the species-poor Polycnemoideae (3 gen./16-18 spp.) and Betoideae (6/16), and the species-rich Amaranthaceae s.str. (c. 70/ c. 1000), Chenopodioidae/Corispermoideae (c. 25/c. 570), and Salsoloideae/Suaedoideae/Salicornioideae (66/c. 700). Of the traditional Chenopodiaceae, the strongly disjunct Betoideae are interpreted as relicts of a formerly widely distributed lineage while

the C/C lineage and the S/S/S lineage show several phases of extensive diversification. Radiations of these two lineages, however, took place in different geographical regions and/or at different times. Using molecular phylogenies, molecular clock dating, lineage through time plots, comparative net diversification rates, genetic diversity in relation to taxon number, and the geographical distribution of extant taxa, phases of diversification and evolutionary stagnation of the three major chenopod lineages are traced in relation to paleoclimatic changes, colonization of new regions, and possible ecological or physiological key-characters.

14.6.2. Amaranthaceae and Chenopodiaceae - one family or two?

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Amaranthaceae and Chenopodiaceae form the most species-rich lineage within Caryophyllales. A widely accepted classification recognizes two families although the merger under Amaranthaceae was proposed recently. Single-gene trees based on *rbcL*, *ndhF*, and *matK/trnK* resolve Amaranthaceae s. str. as monophyletic, but are inconclusive to the position of major chenopodiaceous lineages. Thus, Polycnemoideae either appear sister to Amaranthaceae or first branching in the alliance, and morphological characters in support of either topology can be found. Betoideae are either nested among chenopodiaceous lineages, form a basal polytomy, or are first branching in the alliance. To resolve a more robust phylogeny, a multi-gene approach now combines information from plastid *rbcL*, *matK*, and *ndhF* genes, the *trnK* group II intron, the *atpB-rbcL* spacer, and the mitochondrial *matR* gene. In addition to the combined analysis, phylogenetic signals from different data partitions are compared. Alternative taxonomic treatments are discussed that divide the alliance into one, two, three and four families, respectively.

14.6.3. Study of the genetic diversity within the Salicornieae (Chenopodiaceae) of NW France by means of nuclear and chloroplast DNA markers

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Phylogenetic relationships in members of the Salicornieae tribe, natively occurring in the NW coasts of France, were assessed by using three molecular markers: nuclear ribosomal internal transcribed spacer (ITS), chloroplast *trnL-F* (*trnL-F*) and chloroplast *matK* sequences. Parsimony analysis of the ITS sequences failed to resolve clearly the phylogenetic relationships at the specific level within the genus *Salicornia*. In parallel to phylogenetic studies, a population genetic study was carried out by randomly amplified polymorphic DNAs (RAPD). Analysis of molecular variance (AMOVA) of RAPD fingerprints and neighbour-joining analysis of the populations confirms neither the morphology-based classification at the specific level within the genus *Salicornia*. Both sequencing and RAPD studies were able to differentiate diploid and tetraploid species. On the base of our results, the observed morphological differences do not correspond to different species, rather to "morphotypes", as first suggested by Stace (Stace, 1997). In addition, we confirmed the phylogenetic position of the genus *Arthrocnemum*.

14.6.4. Molecular phylogenetic analysis of american *Atriplex* (Chenopodiaceae)

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A phylogenetic investigation of *Atriplex* (Chenopodiaceae) is underway to examine evolutionary patterns, historical biogeography, life-form shifts, and ecophysiological change in the North American lineages (ca. 62 species), with an emphasis on Californian diversity (ca. 37 species, including eight endemics). Necessary limits on taxonomic and gene sampling in previous analyses have left relationships within *Atriplex* incompletely understood. Expanded molecular phylogenetic sampling of North American members of *Atriplex* suggest that neither *Atriplex*, as traditionally recognized, nor the North American taxa are monophyletic groups. American taxa may represent two distinct lineages with different photosynthetic pathways. American *Atriplex*

taxa with the C3 photosynthetic pathway and some other American C3 Atripliceae taxa may constitute a clade. Life-form evolution in North American Atriplex has been dynamic, with multiple, independent shifts between perennial and annual habits. Exploration of additional taxa and gene regions is being pursued.

14.6.5. Phylogeny and C4 evolution in the genus *Suaeda* (Chenopodiaceae) and related taxa

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The c. 85 taxa of Suaedoideae are important components of halophytic vegetation worldwide and developed different C₄-associated leaf types. In this analysis we deepened our earlier study (Schütze et al. 2003) by extending the sampling. We used comparative sequencing of the nuclear ribosomal ITS region and two non-transcribed chloroplast DNA spacers (*atpB-rbcL* and *psbB-psbH*) to reconstruct phylogenetic relationships and evolution of key characters. The main results are: (1) *Suaeda*, *Borszczowia* and *Alexandra* together form a monophyletic group while *Bienertia* holds a position intermediate to Salicornioideae. (2) *Suaeda* splits into two major subclades: The first consists of the annual C₃ species of sect. *Brezia*, the second contains all other known species. (3) Several taxa of sect. *Brezia* show intraspecific ITS variability which can be used for biogeographic studies. (4) The second subclade contains the sections *Schanginia*, *Borszczowia*, *Suaeda*, *Physophora*, *Schoberia*, *Salsina* and *Glaucae*. C₄ photosynthesis appears to have evolved independently three times in this group, and once in *Bienertia*.

14.6.6. New morphological characters help in classification of Chenopodiaceae

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The diversity of traditional classification schemes in Chenopodiaceae, present at all levels, results from both the scarcity of characters and from uncertainties concerning their significance. To overcome these problems, we started a search for new morphological characters and at the same time reassessed traditional characters on the background of molecular trees (*rbcL*, ITS, *atpB-rbcL*, and *psbB-psbH*). In the contribution some results from Suaedeae, Salsoleae and Camphorosmeae will be presented. New characters were found in particular in leaf and stem anatomy, trichome type, and in cotyledon structure. The anatomical results are supported by molecular data gained from extensive sampling of the respective subfamilies and allow us to suggest refined classifications at the level of tribes, genera and sections. The phylogenetic trees also revealed a tribe-specific distribution of the two subtypes of C₄ photosynthesis, which can likewise be used as taxonomic markers in Chenopodiaceae.

14.6.7. New insights in the systematics of *Chenopodiaceae* s.l. (Chenopodiaceae)

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Tribe *Chenopodiaceae* traditionally housed ca. 7 widely recognized genera. However, in many cases their relationships and generic limits remained obscure. Results of recent morphological, phytogeographical and molecular phylogenetic studies considerably changed our views on circumscription of and natural relations within the tribe, which probably require dramatic taxonomic and nomenclatural rearrangements. Some genera traditionally placed in *Atripliceae* appear to be phylogenetically interspersed with *Chenopodiaceae* s.str. As an example I consider here the case of three clades: *Chenopodium* s.str., *Blitum* (incl. *Monolepis*, *Micromonolepis*, *Scleroblitum* etc.), and *Dysphania* s.l. (incl. glandular-pubescent taxa previously placed in *Chenopodium* or segregated as *Teloxys*, *Roubieva*, etc.). Main trends of morphological evolution and hypothetical dispersal routes of these three lineages are outlined and most promising taxa for molecular phylogenetic studies are identified. Further progress in systematics of the tribe is possible through combination of morphology-based taxonomy, phytogeographical and molecular phylogenetic approaches.

14.7.1. Evolution of fruit epidermal silica bodies in genus *Carex* and tribe Cariceae.

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Since the 1970s, fruit epidermal silica bodies have become an integral part of systematic studies in *Carex* (ca. 2000 spp.) as a tool for circumscribing species and sections. Early studies suggested that these characters were conserved, though conflicts with traditional classifications have since questioned their value. This study re-evaluates their utility by examining character change on recent phylogenies in *Carex* and the Cariceae. Patterns of silica body variation in genus *Uncinia* parallel the positive and negative conclusions drawn from studies in *Carex*. Silica body groups frequently conflict with traditional classification, large taxa cannot be defined, and the morphologies of distant taxa are often identical. However, silica bodies are generally consistent with the circumscription of small, well-defined clades in both genera. *Carex*-wide analyses demonstrate that silica bodies can sometimes be useful for delimiting sections, and they are often ideal for circumscribing species. Problems with homology assessment and a lack of discrete characters indicate that silica bodies are best used for species circumscriptions.

14.7.2. using morphology to reconstruct fine-scale phylogenetic relationships in *Carex* (Cyperaceae): Lessons learned

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Two groups are the subject of ongoing studies to determine the suitability of morphology, s.l. (macromorphology, micromorphology, and anatomy) for phylogenetic reconstruction of *Carex* at the levels of species and section. The first group; sections *Careyanae*, *Granulares*, and *Griseae* (subg. *Carex*); consists of ca. 35 North American and Central American species. The second group, section *Deweyanae* (subg. *Vignea*), is comprised of 6 species of North America and Japan. In combination, anatomy, micromorphology, and macromorphology yield informative data in *Carex* sufficient to diagnose sections and provide highly resolved phylogenetic hypotheses, though support is often low. Any one of these data sets alone usually provides low resolution. The trees reveal significant aspects of character evolution, chromosome number change, and evolution of habitat specificity. For example, reddish coloration of shoot bases is phylogenetically informative, though apparently of four independent origins in the first group. Chromosome number and such environmental parameters as soil calcium concentration often are quite labile in closely related species.

14.7.3. Phylogeny, species-richness and ecological specialization in Cyperaceae tribe Cariceae

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Cyperaceae tribe Cariceae is characterized by both species-richness and habitat diversity. Current phylogenetic hypotheses for this tribe and for particular clades within the Core *Carex* group based on DNA sequence comparisons for at least three gene regions will be used to illustrate niche conservatism for ecologically important traits such as flooding tolerance and shade tolerance as well as niche differentiation between closely related species that co-occur within habitats along gradients of soil pH, soil texture, or water depth. The ability to invade wetland habitats appears to be derived within the tribe and to have evolved only a few times, but in concert with different strategies of clonal growth in different clades and varying levels of species-richness. Clades of predominantly wetland species tend to have broad geographic distribution, often with sister-species on different continents, suggesting recent dispersal. In contrast, species within several early diverging clades are predominantly forest specialists with distinct Asian and North American lineages.

14.7.4. The genus *Anthracoidea* (Basidiomycota, Ustilaginales) - a marker for the evolution of Cariceae?

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The species of the smut genus Anthracoidea are specific parasites on the subfamily Cariceae with great preference on the genus *Carex*. The infection site is restricted to the ovaries and appears to be exactly synchronized to host's flowering period. The fungal life cycle, the host specificity and the ecology of typical Anthracoidea-species will be presented. For the first time molecular data are available for nearly half of the described species. Phylogenetic trees are inferred with neighbor-joining (NJ), maximum parsimony (MP), and Bayesian Markov chain Monte Carlo (MCMC) methods. The results are discussed with respect to the species concept and the traditional subdivision of the genus Anthracoidea into subgenera and sections. Furthermore, our results support the notion that molecular data from the parasitic smut genus Anthracoidea may help interpret different phylogenetic hypotheses of its host species.

14.7.5. Chromosome evolution in tribe Cariceae (Cyperaceae): Comparative studies at several hierarchical levels with emphasis on *Carex* section *Ovales*

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Understanding chromosome number change in sedges (Cyperaceae) is essential to understanding the role of chromosomal evolution in diversification of the family. Phylogenetic reconstructions in tribe Cariceae suggest that chromosomal evolution proceeds neither by stepwise changes nor by a general tendency for chromosome numbers to increase. Phylogenetic data within *Carex* section *Ovales* suggest that chromosome numbers can be phylogenetically patterned at the sectional level, and that there was a significant decrease in chromosome number in the lineage that gave rise to a major clade of eastern North American taxa. There is also weak evidence that sister species are more similar in chromosome number than would be expected if chromosome numbers were randomly distributed. However, chromosome counts appear to be highly labile within species, with no obvious correlations between geography and chromosome number. A model of chromosome change within *Carex* must account for this seeming paradox: an apparently random pattern of chromosome counts within species on one hand, a correlation between phylogeny and chromosome counts on the other.

14.7.6. Relationships, basal taxa, and inflorescence evolution in *Carex* subg. *Vignea* (Cyperaceae) as inferred from non-coding rDNA sequences

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Carex subg. *Vignea* is a distinctive taxon (ca. 300 spp., 28 sections) often characterized by bisexual spikes, distigmatic flowers, and the lack of prophylls. Molecular phylogenetic studies indicate that this subgenus is monophyletic, with the atypical *C. gibba*, with tristigmatic flowers and prophylls, found at its base. Several clades distinguish the remainder of *Vignea*; however, these clades do not correspond to easily defined morphological groups. Many traditionally recognized sections are not supported while others, such as sects. *Ovales*, *Stellulatae*, and *Glareosae*, are upheld. Topological tests reject a hypothesis of separate gynaeandrous/androgynous clades. Species of uncertain subgeneric affinity, sometimes linked to subg. *Vignea*, are variously placed in our analysis. For example, androgynous, unispicate species with distigmatic flowers belong to a clade sister to *Vignea*, whereas dioecious, unispicate species with two stigmas are nested within the subgenus. Species with highly compound inflorescences, often regarded as representing the primitive condition within *Carex*, occupy derived positions in the phylogenetic tree.

14.8.1. Flexibility of the coral-algal symbiosis

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The relationship between corals and algal endosymbionts of the genus *Symbiodinium* (zooxanthellae) has been a key factor in the success of scleractinian (stony) corals as modern reef-builders. The genus *Symbiodinium* comprises an extraordinarily

heterogeneous group of dinoflagellates. So far, eight phylogenetic clades have been identified based on nuclear ribosomal DNA (A-H), and within each clade sub-clades (strains or species) are present. In many cases, the juvenile coral acquires zooxanthellae from the environment, and can associate with a wide range of algal strains. The occurrence of certain *Symbiodinium* types in scleractinian corals is sometimes correlated with the local light and/or temperature environment, even within individual colonies. However, the role of symbiont identity and diversity in terms of fitness of the holobiont (i.e., the host-symbiont partnership) has only been poorly studied. I will discuss the affect of zooxanthella type on holobiont physiology, the flexibility of the host-symbiont association and how this flexibility may be an important mechanism for corals to cope with environmental change.

14.8.2. Coevolutionary patterns and scale: algal selection by lichen fungi.

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Trebouxia is one of the most common genera of green algae that form associations with lichen fungi. Species of *Trebouxia* are unicellular coccoid green algae that reproduce vegetatively in the lichen while sexual reproduction occurs in the free-living state. It is thought that lichen fungi "select" compatible algal partners to form lichen associations. Low selectivity by the fungus promotes lichenization with a variety of algal genotypes or taxa. High selectivity by the fungus limits lichenization with algal partners and the fungus becomes specific to the alga. With high selectivity, and hence specificity, coevolutionary patterns become apparent between lichen symbionts. Coevolutionary patterns may be detected at various scales of taxonomic breadth and geographic distribution. Effective wind dispersal of algae in vegetative propagules of the lichen would ensure distribution of algal genotypes for sexual reproduction of the alga as well as for selection by the lichen fungus.

14.8.3. Effects of human management on the population biology of the red alga *Gracilaria chilensis*.

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The Rhodophyte *Gracilaria chilensis* has been used as a resource for the agar industry for decades in Chile. It occurs in two contrasted environmental conditions: (1) in soft-bottom areas protected from waves, characterised by a high biomass of anchored thalli in sand or mud, or (2) on rocky substratum, characterised by a lower biomass of thalli fixed to the substratum by a holdfast. Management practices range from harvesting soft-bottom natural populations to farming of introduced material.

Reproductive phenology, genetic diversity and epidemiology of epiphytes were compared between managed and non-managed populations all along Chile. Both reproductive maturity and genetic diversity are lower in managed than in non-managed populations. Contrarily, epidemiological surveys show a strong epiphyte load in the farms, whereas epiphytes are in very low frequency in natural hard-bottom populations.

The results are discussed in the context of reproductive investment and genetic resources in *G. chilensis*. Overall, the integration of our results indicate that cultural practices have been rambling and point out the need for a strain selection program.

14.8.4. Species versus carotenoid synthesis: what is known about the genus *Dunaliella* from a systematic perspective?

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In spite of 28 morpho-physiological species of *Dunaliella* recognized by Massyuk in her monograph of the genus, the overall phylogenetic perspective of these taxa, including DNA data, is incomplete. This presentation deals with the major progress done by our research group on two approaches in this topic: (1) reappraisal of the biochemical and physiological attributes employed to separate the genus into 4 sections and the underlying DNA data supporting this classification and (2) comparisons among various strains of *D. salina*, based on total amount of carotenoids, - and -carotene proportion, -carotene isomeric ratio and growth rates under different temperatures, light regimes

and salt concentrations. The main results indicate that (1) there is a correlation between the biochemical and physiological attributes with the genetic diversity (nuclear rDNA ITS-1 and ITS-2 spacers sequences) among taxa at the section level, and (2) significant physiological and molecular variability (ITS sequences + DNA fingerprinting analysis-RAPDs) is found among strains in *D. salina*, results that may be indicative of the presence of more than one species among them.

14.9.1. Ovule fates and the evolution of reproductive diversity

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Floral and inflorescence traits govern a plant's reproductive output and the genetic diversity of its offspring through their effects on pollen and ovules fates. The variety of these fates and their interactions create alternate options for the diversification of reproductive morphology and the mating system. These opportunities are illustrated with an exploration of ovule fates, their phenology and interdependence, focusing on two main topics. First, this analysis explores how the low seed:ovule ratio found in outcrossing species could function to absorb embryo death during development, without reducing fertility. Such reproductive compensation exposes the possibility that seed production can be ovule limited, as well as pollen- or resource-limited, which increases the variety of situations in which production of both selfed and outcrossed seeds maximizes maternal fitness. Second, this analysis considers how the evolution of the same mating system can be accommodated by alternate combinations of floral design and display. These examples illustrate the key role played by ovule (and pollen) fates in the reproductive diversification of angiosperms.

14.9.2. Floral design and the evolution of sexual polymorphisms in *Narcissus*, the daffodil genus

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Narcissus exhibits an unusual diversity of sexual systems, with populations that are monomorphic, dimorphic or trimorphic for style length. Associated with this variation are striking differences among species in floral design and pollination biology. This diversity provides an opportunity to investigate the evolution of sexual polymorphisms, and determine the role that floral morphology plays in promoting transitions among sexual systems. Phylogenetic evidence indicates that stylar monomorphism is ancestral in the genus, with multiple origins of stylar polymorphism, including independent origins of stigma-height dimorphism, distyly and tristily. *Narcissus triandrus* is particularly useful for investigating the evolution of stylar polymorphisms because populations are either dimorphic or trimorphic for style length and exhibit striking patterns of geographical variation in floral morphology. Here we present new empirical data comparing demographic parameters, floral morphology, mating and fertility in dimorphic and trimorphic populations that yield insight into the selective mechanisms maintaining sexual polymorphisms.

14.9.3. Pollen fates and the evolution of pollen packaging in plants

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The efficiency of pollen transfer to conspecific stigmas (PTE) largely governs paternal mating success in plants. Compared to species with granular pollen, orchids and asclepiads realize ten- to hundred-fold higher PTE, largely because their aggregated pollinia reduce pollen wastage during transport. Increased PTE should favour packaging, but this benefit is limited by the reduction in potential mating partners for a given number of flowers, and an increased risk of geitonogamous self-pollination, which causes diminishing returns for increased flower production. Many orchids combine pollen packaging with lack of floral rewards, and thereby achieve high levels of cross-pollination. Asclepiads, which are mostly rewarding, appear to experience high levels of self-insertion of pollinia, but also possess self-incompatibility and flowers with multiple stigmatic entrances that increase opportunities for cross-pollination. Gradual torsion of pollinia after withdrawal from the anther also limit self-pollination effectively in both orchids and asclepiads, especially when pollinators spend long periods foraging on one plant.

14.9.4. Towards the quantitative ecology of outcrossing

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I focus on patterns of flower-to-flower gene dispersal in animal-pollinated plants, which I term 'paternity shadows'. The paternity shadow provides the basis for an accounting system that enables levels of outcrossing to be calculated. Based on these calculations, I speculate about the factors that influence the form of a paternity shadow and its adaptive ideal. Each plant-pollinator combination produces a different paternity shadow whose implications for outcrossing can be calculated, thereby providing a quantitative foundation for a comparative ecology of outcrossing. Based on paternity shadows, I formulate a model to investigate pollinator-mediated gene dispersal in a plant with two pollinator species. My analysis shows that if the plant is under selection for outcrossing, it is selected to favour visits from one of the pollinators; selection need not stringently favour complete specialization, however. This result provides a new explanation for some of the many incompletely specialized flowers in nature.

14.9.5. Population context of pollination

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Plant population characteristics such as size, density, and genetic composition may strongly affect patterns of pollinator visitation, pollen transfer and plant reproductive success. Pollination failure in small, sparse populations can give rise to Allee effects, which may influence population dynamics and mating-system evolution. Frequency-dependent selection caused by interactions with pollinators may contribute to the maintenance of floral polymorphism. In this presentation, I will briefly review recent studies to address the hypotheses that (a) density-dependence of pollination success is stronger in self-incompatible than in self-compatible plants, and (b) density-dependence is negative rather than positive in non-rewarding animal-pollinated plants. I will then widen the perspective, and discuss how variation in the abiotic environment and interactions with herbivores may mould selection on floral characters and effects of population characteristics on plant reproductive success.

14.9.6. Modeling selection on floral characters: conceptual foundations and extensions to pollinator interactions

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Selection drives floral character adaptation. Male and female functions contribute equally to selection in a fundamental and precise sense encapsulated by marginal fitnesses (in optimality models) and selection gradients (in quantitative genetic models). Important features such as hermaphroditism and metamerism emphasize themes of conflict, compromise, and genetic constraint. Coupling plant and pollinator dynamics allows exploration of how floral characters such as reward (e.g., nectar production) or advertisement (e.g., corolla size) evolve. Equally important can be selection for reproductive assurance leading to limited selfing, especially under temporally or spatially variable pollinator service. Such limited selfing influences the structure of genetic variation and the response to selection in ways that are important for floral character adaptation.

14.9.7. Effects of floral characters on flower visits by bumble bees

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Within the genus *Penstemon*, hummingbird-adapted species have evolved repeatedly from hymenopteran-adapted ancestors, with independent convergence in flower morphology toward characters exemplifying the "hummingbird syndrome." In previous work, Castellanos et al. sought to characterize those morphological changes as "pro-bird" or "anti-bee" adaptations by surgically modifying flowers of the bee-adapted *P. strictus* to give them characters more like those of bird-adapted taxa. Castellanos et al. measured pollen removal and receipt, along with short-term choice experiments by bees and birds in flight cage cages. Here, we present improved choice tests using free-foraging animals in the field. Site-faithful bees appear to learn the locations of manipulated

plants and respond to two “bird characters” (constriction of corolla tubes and removal of the landing platforms formed by the lower corolla lobes) by making fewer return visits. The data support the interpretation of those characters as “anti-bee” in function.

14.10.1. The structure of plant Photosystem I - It is the biochemistry

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We determined the crystal structure of the complete photosystem I (PSI) from a higher plant to 4.4 Å resolution. Its intricate structure shows 12 core subunits, 4 different membranal light-harvesting proteins (LHCI) assembled in a half moon shape on one side of the core, 45 transmembrane helices, 167 chlorophylls, 3 Fe-S clusters and 2 phyloquinones. The determined structure of plant photosystem I (PSI) provides the first relatively high-resolution structural model of a supercomplex containing a reaction center and its peripheral antenna (LHCI). The peripheral antenna of PSI (LHCI) is composed of four gene products (Lhca1-4) that are unique among the chlorophyll a/b binding proteins in their pronounced long-wavelength absorbance and in their assembly into dimers. Some of the structural features responsible for the unique properties of LHCI will be described and the advantages of the particular LHCI dimerization mode over monomeric or trimeric forms will be discussed. The interactions between the peripheral antenna will be discussed.

14.10.2. The light harvesting complex II antenna protein CP29 is involved in regulating photosynthetic state transitions

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State 1-State 2 transitions in photosynthetic membranes of plants and green algae involve the functional coupling of phosphorylated Light Harvesting Complexes of photosystem II (LHCII) to photosystem I (PSI). We show that exposure of the unicellular alga *Chlamydomonas reinhardtii* to the State 2 conditions leads to phosphorylation of the minor light harvesting protein CP29 of photosystem II (PSII) at four distinct sites localized at the amino acid residues 6, 16, 32 and 102 of its amino terminus. Mass spectrometric analysis of CP29 phosphorylation in the photosynthetic membranes shows that threonine 16 and serine 102 are phosphorylated exclusively in State 2, while phosphorylation of threonines 6 and 32 may also be detected in State 1-exposed cells. The LHCI-PSI supercomplex isolated from the algae in State 2 is found to contain strongly associated CP29 in phosphorylated form. Electron microscopy and single particle analyses indicate that the binding site is close to the PsaH protein suggesting that phospho-CP29 may act as a linker for the association of phospho-LHCII with PSI. In this way CP29 could facilitate the functional coupling of LHCII to PSI in the conditions favoring light absorption by PSII in addition to its well-documented role as a linker between PSII core proteins and LHCII trimers. Redox-dependent multiple phosphorylation of CP29 is suggested as an integral part of the State transition process in which the structural changes of CP29, induced by reversible phosphorylation, may determine LHCII affinity for either of the two photosystems.

14.10.3. Supercomplexes of photosystem I and antenna proteins in green plants and cyanobacteria

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Photosystem I (PSI) and Photosystem II (PSII) can form supercomplexes with various types of antenna proteins. One such protein is Light-harvesting complex II (LHCII). We have characterized the PSI-LHCII complex from *Arabidopsis* membranes in state 2 by single particle electron microscopy at about 16 Å resolution. PSI binds one trimer at the site of the PsaK subunit. Cyanobacteria express large quantities of the Iron Stress Inducible protein IsiA under iron deficiency. IsiA can assemble into numerous types of single- or double rings surrounding PS I. These supercomplexes are functional in light-harvesting, but empty IsiA rings are effective energy dissipators. Electron microscopy studies on projections of these supercomplexes show that photosystem I

trimers bind 18 IsiA copies in a single ring; whereas monomers may bind up to 35 copies in two rings. This shows that IsiA can form a remarkable large variety of ring-like structures. Work on particles purified from mutants indicates that the PsaF and PsaL subunits facilitate the formation of closed rings around PSI monomers, but are not obligatory components of PSI in the formation of PSI-IsiA supercomplexes.

14.10.4. Molecular mechanisms of light acclimation in plants

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One of the great challenges in plant cell biology is to determine the regulatory mechanisms employed by photosynthetic organisms to adapt to environmental changes. To identify components for cellular signalling during light acclimation, we have applied a chlorophyll fluorescence based screen to identify mutants of *Chlamydomonas reinhardtii* and *Arabidopsis thaliana* that are impaired in redox-mediated chloroplast state transitions. From this mutant collection we identified three new proteins. All three are involved in inter-organell signalling between chloroplast, nucleus and mitochondrion as a response to a changing light environment. Nab1 was identified as an RNA-binding protein essential for the control of post-transcriptional expression of light harvesting antenna proteins. Moc1 was identified as a mitochondrial factor that regulates the composition of the mitochondrial respiratory chain in the light so that it can act as an effective sink for reductant produced by the chloroplast. Arg2 was identified as a second mitochondrial arginase of *Arabidopsis* playing an essential role in regulating the arginine metabolism during light stress.

14.10.5. Structure of photosystem II and its implications

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Photosystem II is a membrane protein complex which catalyses the oxidation of water to molecular oxygen and reducing equivalents. The reaction occurs at a metal centre composed of 4 Mn ions and a Ca ion, is thermodynamically demanding and involves the generation of highly oxidised species. Unavoidable side reactions cause detrimental effects on the protein environment leading to the rapid turnover of the reaction center D1 protein. To understand the mechanisms of water oxidation and D1 turnover structural information is required. Initially the positioning of various protein subunits and their transmembrane helices was elucidated by electron microscopy (1) and more recently a refined structure of the cyanobacterial PSII has been elucidated by X-ray crystallography (2). The implications of these structural studies will be discussed in relationship to the unique facets of PSII function.

1. Barber J (2003) Photosystem II: The engine of life. *Quart. Revs. Biophys.* 36: 71-89

2. Ferreira et al. (2004) *Science* 303: 1831-1838

14.10.6. Bicarbonate requirement for the water-oxidizing complex of Photosystem II

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Strong evidence for bicarbonate (BC) requirement within the water-oxidizing complex (WOC) of photosystem II (PSII) is presented. It is suggested that BC is an integral component of the WOC essential for its re-assembly, function and stability. Comparative studies of electrochemical and functional properties of complexes of Mn(II) and Mn(III) with BC and other carboxylate ligands (acetate, formate) show that the unique capability of BC to initiate re-assembly of the tetramanganese cluster of the WOC from Mn(II) and apo-WOC-PSII can be attributed to formation of electroneutral, easily oxidizable Mn-bicarbonate complexes that serve as building blocks for the WOC. It is suggested [Dismukes, Klimov et al., *PNAS* (2001) 98: 2170] that due to this property BC might have been critical to the evolutionary transition from anoxygenic to oxygenic photosynthesis in the Archean period (>2.2 BYA) (*Supported by the HFSP and the program MCB RAS*).

14.11.1. The hydraulic conductance of plant organs as a major determinant of the water balance of plants

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Our understanding of the water balance of plants has enriched with many recent studies mapping the hydraulic parameters in different plant organs. Plants are commonly viewed as water conductors consisting of two opposite poles (the root and the leaf) with high hydraulic resistance (R) and connected by a water pathway with intrinsically low resistance (the stem). This model is, of course, an oversimplification of plant hydraulics. Both the root and leaf include two water pathways i.e. the nonvascular and the vascular compartment. In both organs, the hydraulic conductance ($K=1/R$) of the nonvascular compartment appears to vary in response to several environmental factors and the K of the vascular compartment can vary greatly due to xylem embolism. A first conclusion is that the hydraulic efficiency of a root or leaf is a close function of the partitioning of R between the vascular and the nonvascular compartment. The stem itself is subject to large impairment due to embolism and may, therefore, contribute tremendously to increase the R of the plant. Recovery from xylem embolism appears to be under metabolic control and is part of the plant water balance regulation.

14.11.2. Distribution of hydraulic conductance within a crown of little-leaf linden (*Tilia cordata*)

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Responses of leaf conductance (g_L) to photosynthetic photon flux density (Q_P), leaf-to-air vapour pressure difference (VPD), bulk leaf water potential (ψ_L) and total hydraulic conductance (G_T) were examined with respect to leaf position in the crown. The studies were performed on intact and detached branches cut from the upper (UC) and lower crown (LC). The UC leaves demonstrated 2.0-2.2 times higher ($P<0.001$) daily maxima of g_L than the LC leaves. Mean G_T was 1.9 times higher ($P<0.001$) for the UC foliage. Distribution of the total resistance: soil to distal branches 41-51%, 25-cm distal branches 10-15%, leaves 39-44%. In LC g_L was constrained both by low light availability and limited water supply. Artificial reduction of hydraulic constraints raised ψ_L and altered g_L less sensitive to changes in both atmospheric and plant inner factors. Stomatal responses to VPD were significantly modified by leaf water status: high ψ_L inverted the g_L versus VPD relationship. The improved water supply increased g_L and E in the LC foliage, but not in the UC foliage. The results support the idea that leaves in the lower canopy are hydraulically more constrained than in the upper canopy.

14.11.3. Xylem integrity and transport in developing grape berries

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Water transport to fleshy fruit generally changes from primarily xylemic to phloemic during development, and in grape this transition occurs abruptly. The current hypothesis to explain this transition, particularly in grape (*Vitis vinifera* L.) berries, is that the xylem conduits lose integrity and become non-functional as a result of post-veraison berry growth. We adapted the pressure plate/pressure membrane apparatus that is commonly used to study soil water characteristics, and reevaluated xylem functionality by establishing a hydrostatic (tension) gradient between the pedicel and a cut surface at the stylar end of the berry. The apoplastic tracer dye, basic fuchsin, moved from the pedicel to throughout the peripheral xylem conduits of the berry mesocarp in samples collected both before and after the transition. In addition, isolated tracheids revealed no evidence of physical disruption. We hypothesize that rather than a loss of xylem function, the transition away from xylem transport is a result of the loss of an appropriate driving force (hydrostatic gradient) in the berry apoplast.

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14.11.4. Dynamic variations in xylem specific conductivity: mechanisms and consequences for water movement through sapwood

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The goal of this study was to determine the magnitude of deviation of trunk sapwood specific conductivity (k_s) from that predicted by Darcy's law when k_s is measured under steady-state vs. non-steady state conditions. In maple and alder, two diffuse porous

species with high pit resistance, and in hemlock and Douglas-fir, two conifer species with bordered pits bearing a torus, k_s was 10-30% higher under steady than non-steady state. In these four species, when the pressure gradient increased from 0.01 MPa m⁻¹ to 0.15 MPa m⁻¹, k_s increased by 25% and 45% under steady state and non-steady state, respectively. There was no significant difference between k_s measured under steady or non-steady state in white oak, a ring porous species with high k_s and low bordered pit resistance, as well as in western redcedar, a conifer species that has a simple bordered pit structure (membrane lacking torus). We hypothesize that the elasticity of pit membrane is the main cause for flow rate to be disproportional to the pressure gradient. This study showed that the common assumption that water flow in trees occurs in the steady state has led to significant errors.

14.11.5. Light and temperature induced variable leaf symplasmic hydraulic conductances in *Juglans regia* L.: are aquaporins involved?

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Leaf hydraulic conductance were measured by perfusion with distilled water. When dark acclimated walnut leaves were exposed to light, a rapid increase in hydraulic conductance was measured. The half time of the response was ca 20 minutes. The light response was strongly temperature dependant, with no response at 5°C and a maximum response at 35°C. Light response was partly or totally inhibited when leaves were perfused with solutions of DMSO or HgCl₂, putative aquaporins inhibitors. In presence of Cycloheximide, a general protein traduction inhibitor, the light response was also inhibited. These results demonstrate that the water pathway in walnut leaves is mostly symplasmic, and that proteins, most likely aquaporins, are involved in the regulation of the hydraulic resistance of this pathway. The functional consequences in terms of water relations and gas exchanges of these results are discussed.

14.11.6. Net salt circulation and cation exchange in the conducting bundles increase xylem water potential and prevent sodium stress in assimilating leaves of the common reed (*Phragmites australis* TRIN ex Steudel) growing in brackish water

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Salt exudation into the root vessels and desalting of the ascending xylem sap increase the water potential in shoots of reed growing in brackish waters. A non-destructive technique has been developed for field measurement of the water potential in xylem vessels of medial internodes. At night, it was found to be higher by about 0.4 MPa than that of the brackish substrate (- 0.6 to - 0.7 MPa at the investigated stand). Cell sap osmotic pressure in leaves taken from brackish stands was not higher by more than 0.2 MPa than that obtained from fresh water stands. Sap flow velocity was found to be > 20 m/h at sunny days. In the ascending sap, osmotic pressure, [Na⁺], and [Cl⁻] decreased from basal to medial internodes, whereas K⁺ remained constant. Assimilating leaves were stressed by a salty aerosol. They contained K⁺ in high concentrations (200-300 mM) but little Na⁺ (< 50 mM), even in plants growing at 300 mM NaCl. K⁺ leaving the leaf blades with the assimilate flow was recirculated already in the leaf sheath and internodes, where it was exchanged by Na⁺. In elder internodes, Na⁺ replaced K⁺ in vacuoles, thus creating a rise of the vertical sodium gradient during the vegetation period.

14.11.7. Freeze-thaw induced embolism in conifers

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Freeze-thaw events can induce embolism in the water transport system of plants. It was hypothesised that bubbles formed in the freezing xylem sap expand during thawing, when they are big and the sap is under tension. Conifers with small tracheids should be resistant to freeze-thaw induced embolism. We studied the induction of embolism by freeze-thaw events in *Picea abies* and *Pinus cembra* via Cryo-SEM observations, conductivity and ultrasonic measurements. Conifers were vulnerable to freeze-thaw induced embolism, when dehydrated and exposed to repeated temperature cycles. Winter at the alpine timberline with intensive

frost drought and many freeze-thaw events therefore caused excessive conductivity losses in twigs. Simulation of these conditions in a temperature chamber also induced embolism in young trees. Cryo-SEM observation revealed clustered air-filled tracheids, whereby cluster sizes increased with the number of temperature cycles. Ultrasonic signals were observed during freezing but not during thawing. We conclude that embolism following freeze-thaw events is not caused by the hypothesised mechanism but probably by air seeding processes.

14.12.1. Bioprospecting of ethno-medicinal plants of Nepal

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Nepal, particularly the Himalayan region, is rich in various plants of medicinal value. The medicinal plants are the backbone of traditional medicine, which is the basis of health care in Nepal for many people. Intelligent bioprospecting of this resource can lead to a long-term scientific, economic and health-promoting benefit to society and country. Important aspects of bioprospecting are acceleration and exploring search for natural products, the use of bioassays relevant to the ethno-medicinal experience as well as to the therapeutic objective and the phytochemical analysis of plants with the aim to identify bioactive components. It is absolutely necessary to accompany bioprospecting by strengthening efforts to conserve the natural resources. The results of an interdisciplinary project between Nepalese and German groups and financed by the Volkswagen foundation demonstrate the high potential of several plants from the Himalayan region for the production of compounds with unique structures and with interesting bioactivities, especially for the treatment of infectious diseases.

14.12.2. Medicinal plants and their management efforts in Nepal

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Nepal is rich in biological resources in general and medicinal plants resources in particular. There are over one thousand plant species of medicinal importance, and around one hundred plant species are used in various drug preparations. Medicinal plants of temperate and alpine regions are of high commercial value, and these resources are either unexplored or over-exploited and illegally traded across the border. It is estimated that every year 20,000 t of the medicinal and aromatic plants (MAPs) are collected from Nepal, however about 90 % of the collection are exported in the raw form mainly to India. Only 10 % of the MAPs collected are processed in the country and exported to third world countries. In the last few years, government and private sectors in Nepal have initiated some efforts to manage medicinal plants. The present paper deals with the status of medicinal plants, management efforts and the analysis of practices related to MAPs.

14.12.3. Politics of biodiversity conservation for rural livelihoods in the Himalayas: An overview of national policies in Nepal

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This study was carried out reviewing and analysing political arena, policies, legal instruments and practices that are in use for the conservation of biological diversity and promotion of the livelihoods of the ordinary people in Nepal, the country which is situated in the Himalayan region and whose over 80 % total land area is comprised of mountains and hills. This study aimed reviewing policies and practices for biodiversity conservation and livelihoods in Nepal. Despite several efforts, the biodiversity resources sustaining livelihoods of the majority of poverty trodden people are at risk. It is due to lack of appropriate policies and enforcement mechanisms, in addition to several generic causes such as poverty, over exploitation, habitat destruction, invasion by alien species, poor governance, etc. The study recommends overcoming the threats to biodiversity through active participation of the local people. The national policies should entrust ownership,

control and management of the resources to the users based on the principles of biodemocracy, human rights and social justice.

14.12.4. Bioprospecting of ethnomedicinal plants of Himalayas: Conservation and sustainable use in Manang, Nepal

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 Tribhuvan University, Kathmandu, Nepal.

Himalayan medicinal plants are globally unique but threatened due to overexploitation. Research applicable under bioprospecting of ethnomedicinal plants is lacking for Nepal. We are working on bioprospecting of medicinal plants of Manang for conservation and sustainable use with objectives to: (a) document medicinal plants used by the local communities and Tibetan healers "amchis", (b) carry out bioassay test to validate the indigenous knowledge, and (c) address issues associated with transfer of indigenous knowledge. Our observations revealed that there has been immense potentiality of bioprospecting of medicinal plants. Antimicrobial testing of 40 valuable medicinal plants by using the disc diffusion method was carried out with three different solvents in different bacteria and fungi to validate the indigenous knowledge. Amchis are respected as a social leader contributing in conservation; but the greatest threat of declining their knowledge and profession are lack of apprentices.

14.13.1. Considerations on biodiversity prospecting and utilization for human welfare

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Agro -biodiversity prospecting has resulted in the selection and improvement of economic plant species for human prosperity over the millennia. Developed countries have continued to benefit from PGR introduced from the gene rich developing countries. When Plant Breeders Rights and IPRs began to be implemented by developed countries, the developing countries started raising ethical issues, demanding equity and benefit sharing using their genetic resources.

Recognizing that unrestricted access to PGR is essential for continued human welfare, the presentation summarizes these historical developments and examines the (i) ethical and equity issues; (ii) elements of CBD, PBRs, TRIPS, FAO International Seed Treaty and national legislations; (iii) FAO Code of Conduct on Plant Germplasm collecting and use; (iv) initiatives of Global Crop Diversity Trust for conservation and utilization of PGR *in prosperity*, and discusses merits /demerits and limitations of alternate mechanisms for benefit sharing, including material transfer agreements, between the providers and users of PGRs.

14.13.2. Issues on Access and Benefit Sharing (ABS) of indigenous knowledge v/s bioresources

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Genetic resources constitute an integral component of biological diversity. The prospects of exploring biodiversity for new medicines, foods, crops, insecticides, pesticides and other commercially valuable genetic and biological products and processes are booming, due to the rapid development in Biotechnology- Herbal Technology and Information Technology. And, this exploring of biodiversity for commercially valuable genetic and biochemical resources is termed as "bioprospecting" which is pursued at three levels viz. chemical prospecting, gene prospecting and bionic prospecting. The paper discusses the recent trends in bioprospecting that necessitate an ever-increasing demand for access to genetic resources and traditional knowledge available in *in situ* and *ex situ* sources. These trends have also triggered conflicting interests and common concerns among all stakeholder states, communities involved in conservation, sustainable utilization, and documentation of genetic resources and traditional knowledge at local, national, regional and global levels.

14.13.3. The Global Crop Diversity Trust: supporting conservation for the welfare of current and future generations

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Ex situ collections provide a safety net for restoring agriculture after disasters and provide a reservoir of readily accessible, well-

characterized genetic diversity for crop improvement. However, genetic diversity is under threat not only in the field but also in genebanks that are meant to be safe havens. The 1996 Global Plan of Action, calls for a rational global system of *ex situ* collections and several recent initiatives are laying a foundation for this. Significant among them is the recent creation, by FAO and IPGRI, of the Global Crop Diversity Trust - an international public-private fund that, as part of the funding strategy of the International Treaty on PGRFA, aims to raise a non-wasting endowment of US\$260 million. Proceeds from the endowment, together with other funding sources, will finance the salvaging and upgrading of the world's key national and international crop germplasm collections and help provide for their maintenance *in perpetuity*, for the ultimate benefit of all humanity. The Trust is currently developing regional and crop conservation strategies to guide the allocation of resources to the most important and needy collections.

14.13.4. Bioprospecting at the community level: An integral component of sustainable ecosystems

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The theme of this presentation is to remind us that biodiversity prospecting is a major activity by resource-poor people to meet their sustainable needs. Bioresources may also be exploited by others for business purposes. Bioprospecting can have negative or positive impacts on biodiversity whether applied by local people or by external collectors with commercial or scientific motives. How farmers and local communities manage their genetic resources, we will draw upon a 6-year study of an old, complex agroecosystem of Mexico, called the milpa. An integrated research program, including socio-economics, ethnobotany, gene flow between crops and their progenitors, and farmer management of maize, squash, and bean landraces and quasi-domestic wild species (quelites) showed how the milpa system is evolving to meet the needs of local households and communities. Ideas for sustaining the milpa agroecosystem with appropriate bioprospecting and translocation of biological resources will be offered.

14.13.5. Ethical issues related to agricultural diversity prospecting and use

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The relationship between human culture and agricultural biodiversity, as opposed to wider biodiversity, is a close one -- humans played a crucial role in the evolution of agricultural genetic resources and these resources, in turn, are crucial to livelihood strategies, particularly of poor peoples. Agricultural biodiversity and related knowledge have been considered a public good. Despite their importance, agricultural diversity is seriously threatened, notwithstanding conservation efforts worldwide. Ethical considerations regarding prospecting, researching and using agricultural biodiversity and related information are presented. Unrestricted access for breeding purposes is very important but the access conditions should also adequately recognize and reflect contributions local communities and farmers have made to create and nurture these resources. Ethical principles to be followed when involving local people and their genetic resources in research activities or when obtaining access are presented, including self-determination, inalienability and traditional guardianship, prior informed consent and veto, and confidentiality.

14.13.6. Genetics resources as public goods : exchange and benefit sharing under the international treaty on plant genetic resources for food and agriculture

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The International Treaty on Plant Genetic Resources for Food and Agriculture, adopted in 2001 under the auspices of the Food and Agriculture Organization, establishes a Multilateral System (MS) for facilitated access to genetic resources and benefit-sharing for 35 food crops and 29 forage genera. Under the MS, Plant Genetic Resources for Food and Agriculture can be accessed to and exchanged free of charge if they are to be solely used for research, breeding or training purposes. Benefits under the MS are

not attributed directly to the country supplying PGRFA on a bilateral basis, but they are shared multilaterally. While the Treaty is compatible with the international rules on intellectual property, it offers a model for research and conservation of global public goods that may counterbalance current trends towards privatization of biological resources and its associated knowledge.

14.13.7. Access and Benefit Sharing of Plant Genetic Resources: Aspects of Equity and Ethics

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Both fundamental and applied botanists need access to research materials. Uncertainty on how to apply the principles laid down in the Convention on Biological Diversity (CBD), entered into force in 1994, and the International Treaty on Plant Genetic Resources for Food and Agriculture (IT PGRFA), entered into force in 2004, hampers the transfer of biological materials for research, whether pure or applied. A standard Material Transfer Agreement as developed for the Multilateral System of Access and Benefit Sharing of the IT PGRFA would facilitate and probably foster the exchange of materials. In addition, the Bonn Guidelines for Access and Benefit Sharing of the CBD (adopted in 2002) contain many elements that help implementation of the CBD.

It is suggested that responsibilities and rights governing germplasm exchange may depend on the research goals; that rules should be unambiguous, and that material transfer agreements should specify the applicable access and benefit regime. Information exchange, capacity building and transfer of technology next to community development may be major mechanisms of benefit sharing.

Poster Presentations

P0001. Development, transferability and screening of nuclear microsatellites in *Cupressus* ssp.

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Cupressus sempervirens L. characterises the landscape in most Mediterranean countries. Since antiquity, cypress has been extensively cultivated far beyond its natural geographic range. Recently, polymorphic microsatellites were developed through the construction of an enriched library, for studying the distribution of diversity, to monitor gene flow, to identify hybrids, and to construct genetic maps. Initially, the level of variation was tested on a total of 24 individuals from four different *C. sempervirens* populations. High level of allelic diversity was found with a number of alleles ranging from 2 to 13. The new primers were tested in 38 *Cupressus* species. For some SSRs, the transferability rate was higher than 85%. Interestingly, the transferability success rate decreases from Mediterranean-, to Asian-, to Central- and North-American species. Distribution of diversity was analysed in eleven *C. sempervirens* populations sampled in Italy, and compared with that of some populations from Turkey. Significant differentiation between the two groups was estimated. Discussion about the possible origin of the Italian populations is reported.

P0002. Determination of ploidy level on the collected natural hybrids of citrus in north of Iran

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It is difficult to observe the chromosomes of citrus in detail due to their small size. Therefore, many cytologists have attempted to improve the techniques for chromosome counts in citrus. In present study Squash technique is used for citrus chromosome counting and distinguishing the ploidy level of natural hybrids of citrus which established in Kotra citrus collection. In this method, the open-pollinated seeds of 25 natural hybrid ripen fruits are washed and sterilized by 1% solution of Sodium Hypochloride. The seed coats are removed (for faster and better germination) and planted in clean petridishes at 25 °C. After germination, one centimeter of root tips detached and following steps were carried : The plant

material used were the 25 natural hybrids of citrus contain : Adeb, Atabakee, Bakraee, Syavaraz(no. 1,2,3 & 4), She-mahalle, Moallem-kooh, Mino, Hybrids no.1,2,3,4,5,6,7,8,9,10,11,12&13, Limou-golabee-amole(Mina) and Limou-astaraee. Eighteen chromosomes (diploid) were observed in all the natural hybrids used.

P0003. Genetic analysis of apomixis in *Taraxacum* (dandelion; Asteraceae)

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Apomixis in the genus *Taraxacum* is represented by meiotic diplospory. It is confined to polyploids, whereas diploids are always sexuals. For apomictic seed formation three steps are essential: diplospory, parthenogenesis and (autonomous) endosperm development. We analysed 194 offspring plants from the *sexual* x *apomict* cross. Segregation of apomixis as a whole suggested three independent dominant genes. *DIP* and *PAR* appeared to be inherited as dominant genes, whereas our data on *END* does not fit to dominant model. Segregation distortion from expected Mendelian ratios was detected for several SSR markers that were linked to apomixis elements (*DIP*, *PAR*). This can be explained (along with an absence of diploid apomicts) by accumulation of deleterious mutations on the apomictic chromosomal segments resulting from repeated hybridisation cycles in long time-scale. First FISH experiments with BACs containing markers linked to diplospory showed higher proportion of repetitive sequences in apomictic genome with clustering in the presumed diplosporic chromosomal segment indicating a high mutational load.

P0004. Nucellar embryo formation time and their further development in five citrus rootstocks

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In this trial the stages of embryo development and the time of polyembryony were studied via microscopical analysis in five citrus rootstocks (Sour orange, Flying dragon, Citromelo, Citrange and Shell mohalle). The fruits were harvested from expected genotypes at 35, 50, 65, 80, 95, 110 and 125 days after pollination (DAP). Diameter and weight of fruits were measured and then seeds were extracted and fixed in FAA. After cross section with Parafin method and staining with Safranin- Fast green the number of ovules per seed were determined. The experimental design was RCD with three replications, each replication consisted of ten seeds. Results showed that none of seeds in 35 DAP had any sort of embryo. Embryos at fifty DAP were in globular shape and at later stages the seeds had one or more developed embryos. The first embryo that develop from globular shape to normal and mature embryo is usually zygotic embryo and recognizing that from nucellar embryos might have great impact in breeding of citrus species. In this study the genotype and seed age significantly affected the phenomenon of polyembryony as well as diameter and weight of fruits

P0005. Using of RAPD markers for identification of nucellar and zygotic progenies in citrus rootstocks breeding program

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Because of abundant polyembryony, citrus breeding need very time and costs. For this results, identification of zygotic and nucellar embryo is very difficult. This Trial carried out for identification of zygotic and nucellar progeny in hybridization between some citrus genotypes as sour orange (*Citrus aurantium*) as male parent and *poncirus trifoliata* as female parent Total DNA was extracted from well grown leaves of parents and progenies. Only seven primers out of 24 primers showed polymorphism after RAPD analysis. Nucellar progenies showed the same RAPD profile as female parent because their origin are from female parent but zygotic progenies showed bands from both female and pollen parents. RAPD markers as OPM04-3000, OPM04-5000, OPM11-650, OPM11-850, OPM14-1200, OPM14-1650, OPM14-1800 and OPM18-1650 were able to discriminate between zygotic and nucellar embryos

P0006. Regulation of apoptosis processes in embryogenic calli of wheat and barley

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Regulation of apoptosis or programmed cell death (PCD) by the use of phytohormones, organic and stress substances has been developed for wheat and barley embryogenic calli. It was established, that increase of 2,4-D concentration up to 5,0 -7,0 mg/l as well as the 500 mg/l proline addition are accompanied with the intensification of PCD which leading to embryo abortion. 1,0 mg/l BAP and 1000 mg/l casein hydrolyzate reduced PCD level, removed embryo blockation and stimulated their differentiation. Increase the ABA concentration up to 1,0 mg/l lead to the total degradation of embryoids cells by PCD pathway. Presence of moderate concentrations of 0,5% NaCl increased PCD level. At the same time with PCD high concentration of NaCl (1,0%) caused the deeper destructive processes, necrosis. Two types of PCD have been revealed by the use of light and electron microscopies: goings on through the plasmolysis and without plasmolysis (cell wall localization of cytoplasm). The plasmolysis type PCD finding in case of embryo disintegration to death cells and single embryogenic cells. PCD going without plasmolysis of cells takes part in differentiation processes.

P0007. *Fusarium* mycotoxins can inhibit programmed cell death (PCD) in *Arabidopsis thaliana*

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The *Fusarium* genus of fungi can be responsible for commercially devastating crop diseases. Apart from severely reducing yield they also produce damaging mycotoxins that are major food poisoning constituents. Two such groups of mycotoxins are the tricothecenes and the fumonisins. Deoxynivalenol (DON) is the most prevalent tricothecene found in crops for food and feed production. It is known that like other protein synthesis inhibitors (e.g. cycloheximide - CHX); DON induces programmed cell death (PCD) in animal cells. Here we show; using an *Arabidopsis* cell system, not only that DON does not induce PCD; it inhibits the PCD induced by other stimuli. Fumonisin B₁ (FB₁) is the most prevalent subspecies of the mycotoxins produced by *Fusarium verticillioides*. It is known to cause PCD in both plants and animals. Here we show that high levels of FB₁ can induce PCD, whereas low levels partially block the progression of cell death in *Arabidopsis*. Because the modes of action of FB₁ and DON are known, our results reveal aspects of the mechanism of PCD in plants.

P0008. Genomic and proteomic analyses of mitochondria of *Arabidopsis* cells undergoing cell death

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Harpin is a type III elicitor, secreted by bacteria such as *Pseudomonas syringae*. Previously we reported that the elicited hypersensitive response (HR) is accompanied by inactivation of mitochondria in *Arabidopsis* cells. We observed a loss of mitochondrial membrane potential and of ATP synthesis. Harpin caused a rapid cytochrome c release from mitochondria into cytosol and a strong induction of protecting or scavenging systems such as AOX and small heat shock proteins. Due to the inactivating effect on mitochondrial functions, we surveyed genetic and proteomic programs of mitochondria behind harpin induced apoptosis. Changes in mitochondrial gene expression were monitored within 24 hours after treatment. We found oppositional behaviour in mitochondrial and global gene regulation. Proteome analysis of mitochondrial fractions confirmed an activating effect on mitochondrial enzymes on protein level. A comparison of genomic and proteomic data sets will be presented and discussed.

P0009. H₂O₂ and ceramide induced programmed cell death in *Arabidopsis* cell cultures requires protein synthesis

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Programmed Cell Death (PCD) involves a series of events in which the cell directs its own demise. It depends upon the active

participation of the dying cell and is gene regulated. PCD is believed to be essential for normal plant development and defence responses. However, very little is known about the regulation of PCD in plants, although *de novo* protein synthesis appears to play an important role in this process. Hydrogen peroxide (H_2O_2) has been established as a key molecule in plant PCD and stress responses. It has been shown to activate numerous signalling cascades resulting in PCD, necrosis and changes in gene expression. Ceramides are bioactive lipids that have been shown to act as second messengers and regulate cellular homeostasis in mammalian cells. Recent research has shown that ceramides can induce cell death in plants. Here, we show the results of inhibiting protein synthesis using cycloheximide and actinomycin D in *Arabidopsis* cell cultures exposed to two different PCD inducing treatments, H_2O_2 and C_2 ceramide.

P0010. DNA Marker for Sex Identification of Papaya (*Carica papaya* L.).

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Papaya (*Carica papaya* L.) is a dioecious plant which cannot use X/Y chromosome system to indicate sex difference. For this reason, the polymerase chain reaction (PCR) with T12 and W11 primers designed from *Sex1* locus that regulated sex expression in papaya, was performed and showed the amplified products of 838 and 832 bp size, respectively, in male and hermaphrodite, but not in female. The identification accuracy of the reaction for sex-specific DNA was 96.97 % from 66 samples in total for both T12 and W11 primer. Amplified fragments sequence analysis demonstrated no difference between male and hermaphrodite with T12 primer, but with W11 primer there was two different bases at 412 and 474 positions of reverse strand between male and hermaphrodite. According to the base difference, the Papaya Hermaphrodite Specific (PHS) primer was designed for hermaphrodite Kheak-dum cultivar. The PCR with W11 and PHS primers showed two different bands of 832 bp for male and 440 bp for hermaphrodite, but no band for female. Under the PCR condition, W11 primer with forward and reverse strand and PHS primer only with reverse strand must be added in one step at the beginning of reaction.

P0011. Cell death induced by hypoxia in the roots

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We studied an effect of hypoxia on the roots of plants, especially focusing on profiles of cell death. When young seedlings of *Brassica rapa* were placed in hypoxic water, the number of roots ceased their growth increased as the exposure time was prolonged. Evans blue staining suggested that the cell death began at the distal elongation zone (DEZ) of roots and then expanded to the neighboring regions to the DEZ. DNA laddering was faintly detected for plants exposed to hypoxia. This may reflect an occasional finding of cells which exhibited a positive reaction to TUNEL assay. Electron microscopy revealed that organelles such as plastids and mitochondria first began to bloat and then inner cell structures disappeared finally leaving their envelopes. As for nuclei, the nucleolar segregation became prominent in a short time exposure to a hypoxic condition and prolonged exposure rendered chromatin aggregate. On the other hand, meristematic cells first died under a hypoxic condition for *Vicia faba* roots. In particular, clear DNA laddering was detected. Thus, profiles of cell death may vary with species and tissues.

P0012. In vitro propagation of Cupressus sempervirens grown in Guilan, IRAN.

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Poster, tonkabon, Islamic Republic of Iran.

The conventional methods of asexual plant propagation of this species have not been successful so far. Small pieces of stem tips of 5-6 years old *C. sempervirens* (0.2-0.5 mm long) with one to three needles fascicles on each, were excised from the plants and after surface sterilization, samples were cultured on a Murashige & Skoog (MS) medium and a Revised MS medium supplemented with different concentrations of BAP, Kin and IBA. The cultures were then incubated in a climate chamber at temperatures of 25°C (day) and 15°C (night) and 12 hours light at 2000-2500 Lux with 75% humidity.

The best stimulation of callus growth occurred on cultures of Rev. MS medium when supplemented with BAP=0.2 mg/l, Kin=0.1 mg/l and IBA=0.2 mg. Callus formation, after several re-culturings became friable and increased in volume when supplemented with BAP=0.2 mg/l and Kin=0.5 mg/l. Adventitious buds were formed on the callus with green needles, usually found on juvenile stages of parent plants.

P0013. Ultrastructure of induced callose deposits is related to the nature of the elicitor

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Onion inner epidermal cells form locally separated callose deposits on their cell walls upon stimulation. Potent elicitors are, e. g., heavy metals, fungal toxins, or, as recently discovered, nitric oxide. The depositions induced by copper and by nitric oxide are structurally different. As light or fluorescence microscopy shows, copper-induced callose is very dense with almost no structural details, whereas nitric oxide-induced callose deposits are more loose or spongy aggregates often surrounded by a thick protoplasmic layer rich in organelles. The different structure is also seen by transmission electron microscopy. Copper-induced callose appears more or less homogeneous and translucent and the plasma membrane is clearly separated and smooth, whereas in nitric oxide-induced callose deposits many membranous whorls as well as cytoplasmic remnants are included, and the boundary between callose and plasma membrane is extremely furrowed. This is an indication that in downstream signalling as well as in callose plaque formation plant cells are able to distinguish chemical stimuli.

P0014. Distribution of Cytokinins in the Fertile Thallus of the Fresh Water Alga Chara contraria A.Br.

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The results of studies and quantitative characteristics of cytokinins in leaf rings from various parts of the fertile thallus of *Chara* have been described. The subject of studies was the fresh-water *CHARA CONTRARIA*. Leaves of different parts of the alga fertile thallus were found to have different ratios of zeatin Z, ZR, iP and iPA. Of all found cytokinins the highest level of ZR was detected in upper young leaves while levels of Z, iP and iPA were almost similar. In leaves from the thallus middle part levels of ZP, iP and iPA were significantly lower while the content of Z remained the same. Levels of all forms of cytokinins in the lower leaves of the alga were the lowest. It appears that such distribution of cytokinins in leaves from various parts of the *Chara* thallus is related not only with the cell growth processes (division, elongation) but also depends on the formation and development of generative organs. Moreover, in nodes of upper leaves there are located oogonium and antheridium, in middle ones - green oogonium with fertilized eggcell, in lower ones - mature red antheridium.

P0015. Bird-Flower Interactions And Pollination In Some Plant Species Of Eastern Ghats Forests Of Andhra Pradesh, India

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The floral characteristics of *Bombax ceiba*, *Firmiana colorata*, *Erythrina suberosa*, *E. variegata*, *E. variegata* var. *orientalis*, *Bauhinia variegata*, *Careya arborea*, *Alangium salviifolium*, *Gmelina arborea*, *Spathodea campanulata* (trees), *Butea superba* (woody climber), *Helicteres isora*, *Woodfordia floribunda* (woody shrub), *Leonotis nepetifolia* (annual herb) and *Dendrophthoe falcata* (tree parasite) were examined in relation to their flower visitors for pollination. The study showed that *F. colorata*, *E. suberosa*, *E. variegata*, *E. variegata* var. *orientalis*, *B. superba*, *W. floribunda*, *L. nepetifolia* and *D. falcata* are strictly ornithophilous and pollinated by passerine and non-passerine birds while the other species are partly ornithophilous. The dry season bloomers play a vital role in sustaining birds, bats and bees by providing during dry season. Some birds damage the flowers and squirrels and monkeys consume them while drinking nectar, effecting the reproductive success of some plants. The study suggests that there is an evolutionary relationship between some plant species and passerine birds for the benefit of both partners.

P0016. Characterization of the effect of the Fusarium mycotoxin zearalenone in *Arabidopsis thaliana*U. Werner¹, F. Berthiller², R. Schuhmacher², R. Krška², G. Adam¹, M. Hauser¹;¹University of Bodenkultur, Vienna, Austria. ²IFA, Tulln, Austria.

Zearalenone (ZON), also known as F2 toxin, is a nonsteroidal estrogenic mycotoxin produced by plant pathogenic *Fusarium* species responsible for diseases in cereal crops. ZON is one of the most potent xenoestrogens and therefore of particular interest to human and domestic animal health. Although ZON may serve as potential fungal virulence factor, its effect in plants is not well characterized to date. To identify the molecular mechanism of ZON's action on plant development and defense we used the model plant *Arabidopsis*. Our results show that ZON inhibits root elongation in a dose dependent manner, antagonizes ethylene's effect on radial cell expansion in CELLULOSE SYNTHASE 3 mutants and interferes with (oxidative) stress responses and cell wall modification/reinforcement. In *Arabidopsis* ZON is rapidly metabolized into zearalenol, ZON-4-O-glucoside and ZON-4-O-sulfate and later two metabolites are partly secreted. Based on our physiological, genetic and molecular results, we propose a model for the mechanism of ZON's mode of action in plants.

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P0017. Physiological effects of ethylene on biochemical parameters of *Crocus sativus* L.

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Despite its simple two-carbon structure, the olefin ethylene is a potent modulator of plant growth and development. The plant hormone ethylene is involved in many aspects of the plant life cycle, including seed germination, flowering, flower senescence and fruit ripening. We have investigated the effect of ethylene on corms of saffron (*Crocus sativus* L.). Corms were treated with varying concentrations (0, 25, 50, 100, 200 µl/l) of ethylene during various stages of growth and were cultured in greenhouse as well as field conditions. The results revealed that ethylene treatment increased content of reducing sugars in corms, roots and leaves, whereas decreased the content of soluble proteins in corms, roots and leaves during various stages of growth of corms. Electrophoretic profile of proteins in the ethylene treated corms revealed that the number and content of protein bands were different when compared to untreated corms. In this study, use of ethylene concentration @ 100µl/l in the month of July had best effect on flowering. Relationships between ethylene concentration, flowering percentage, protein and sugar levels and stages of growth of plant is discussed.

P0018. Delay in the period G₂ and development of chromosomal aberrations caused to the damage in the DNA induced by 5-aminouracil in higher plant cellsL. B. Marciano¹, A. Del Campo¹, M. Bracho¹, J. Guíñez¹, C. De la Torre²;¹Facultad Experimental de Ciencias., Maracaibo, Venezuela, ²Centro de Investigaciones Biológicas (CSIC), Madrid, Spain.**Abstract**

This work evaluated the qualitative and quantitative cellular changes induced by treatment with 5-aminouracil (5-AU) in plant cells in relation to DNA damage. As biological material, *Allium cepa* L. root tips were used, grown in filtered water, in darkness, with aeration at constant temperature of 25 °C ± 0.5. When the roots were 2-3 cm long, were submerged in 5-AU (5 × 10⁻⁴ M) solutions. Roots were separated from the bulb and fixed in a mixture of glacial acetic acid - ethanol (3:1 v/v). Afterward, the meristematic tissues were dehydrated in serial alcohol concentrations, stained by the Feulgen technique. Results showed a G₂ duration of 4 hours, with a delay of 1.8 hours for the beginning of prophase in comparison with the controls and inducing of aberrant metaphases, anaphases and telophases, indicating that the negative G₂ control was weakened during the 5-AU treatment. Settles down the existence of G₂ checkpoints in the cell population studied, which when being surpassed, allowed the development of chromosomal aberrations and cellular instability.

P0019. *Arabidopsis* EVER SHORTER TELOMERE 1 (EST1) homologue is required for exit from meiosisS. Akimcheva¹, R. Idol², J. Puizina¹, N. Riehs¹, D. Schweizer¹, D. Shippen², K. Riha¹;¹Gregor Mendel Institute, Austrian Academy of Sciences, Vienna, Austria, ²Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX, United States.

Cell cycle is composed of a chain of events that ensure duplication and equal distribution of genetic material in daughter cells. Chromosome segregation in mitosis requires activation of the anaphase promoting complex, which permits initiation of anaphase via destructing sister chromatid cohesion, and exit from mitosis by degrading mitotic cyclins. We identified and characterized novel *Arabidopsis* gene that is essential for the exit from meiosis. The gene encodes a protein that contains evolutionary conserved EST1 domain. *Arabidopsis* mutants deficient for the protein exhibit severe growth defects and are sterile. Cytological analysis of meiosis in mutant plants revealed that the sterility is caused by a cell cycle arrest at the anaphase II/telophase II transition. Arrested meiocytes contain separated chromatids that are randomly distributed throughout the cell and only very slowly decondense. We propose that mutants fail to exit meiosis likely due to an insufficient degradation of mitotic cyclins. Thus the *Arabidopsis* Est1 protein represents a novel conserved factor that is required for cell cycle progression.

P0020. Visualization and characterization of large-insert T-DNAs in transgenic riceG. Suzuki¹, A. Nakano¹, M. Yamamoto², Y. Mukai¹;¹Division of Natural Science, Osaka Kyoiku University, Kashiwara, Japan, ²Faculty of Health Sciences for Welfare, Kansai University of Welfare Sciences, Kashiwara, Japan.

Introduction of large-DNA fragments into cereals by *Agrobacterium*-mediated transformation is a useful technique for map-based cloning and molecular breeding. However, little is known about the genomic organization and stability of large foreign fragments in plants. In this study, we produced transgenic rice plants by *Agrobacterium*-mediated transformation with large-insert T-DNA containing a 92-kb genomic region of wheat. The structures of the T-DNAs in four independent transgenic lines were visualized by fluorescence in situ hybridization on extended DNA fibers (fiber FISH). By using this cytogenetic technique, we showed that rearrangements of the large-insert T-DNA, such as duplication, deletion and insertion, have occurred in all four lines. Our analysis suggested that the large T-DNAs tend to be rearranged in transgenic rice plants. In addition, significant deletion in the large-insert DNA was also observed in *Agrobacterium*. It is important that the large-insert clones must be carefully constructed to be as short as possible, and the constructs should be checked for rearrangements in *Agrobacterium* before using them for plant transformation.

P0021. Effective Mutagenesis of *Arabidopsis* by Heavy Ion Beam-Irradiation

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Arabidopsis researches frequently include the genetic approach, so efficient, convenient, and safe methods for mutagenesis are required. Currently, the most popular method for in house mutagenesis is application of EMS. Although this method is very effective, its base substitution-type mutations often gives leaky mutants with residual gene functions, leading some difficulty in understanding the corresponding gene functions. Heavy ion beam generated by accelerators gives highest energy transfer rates among known radiation-based mutagenesis methods including X ray, gamma ray, fast neutron, electron and proton irradiation. This feature is thought to give high frequency of the double strand break of genomic DNA and resultant short deletions, resulting frame shift-type mutations. At RIKEN Accelerator Research Facility (RARF, <http://www.rarf.riken.go.jp/index-e.html>), we have optimized conditions for effective mutagenesis of *Arabidopsis* regarding to ion species and irradiation dose, and achieved comparable mutation rates to the method with EMS.

P0022. Genome instabilities in *Arabidopsis* studied by means of molecular cytogeneticsP. Mokros¹, J. Siroky¹, K. Riha²;¹Institute of Biophysics CAS, Brno, Czech Republic, ²Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria.

Genome integrity is a vital condition for the cell to maintain genetic information and to transfer it to daughter cells by cellular divisions. We study a number of mutants in *Arabidopsis* that are defective in

reparation of DNA breaks, cell cycle progression, and telomere maintenance. Frequent event during the healing of genome damage is fusion of chromosomes via the mechanisms of homologous recombination or non-homologous DNA end joining. To trace the chromosome fusion events we developed a cytological approach employing fluorescent *in situ* hybridization. Using chromosome-specific BAC-derived probes in multicolour *in situ* hybridization experiments particular chromosomes and/or chromosome loci can be accurately assessed within the place of chromosome fusion. Global genome alterations, including aneuploidy, translocations, chromosome deletions and fusions were detected in telomerase-null *Arabidopsis* plants. In *Arabidopsis* deficient in a major repair complex (Mre11), the presence of anaphase bridges and multiple acentric chromosome fragments were detected together with aberrant bivalent formation during meiotic prophase I.

P0023. Which characteristics of the division and of the expansion of maize epidermal leaf cells are intrinsic to the genotype?

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The leaf elongation rate (*LER*) does vary with the position of the leaf on the stem. This study aims to identify if some components of *LER* are independent of the position, thus defining intrinsic characteristics of a genotype. *LER* thermal time course of leaves 4 to 9 was measured for three maize genotypes. The spatial profiles of velocity and of epidermal cell length were measured at 3 leaf positions per genotype. Data were analysed in terms of the spatial organisation of the growth zone, cell-element-specific and cell-group specific characteristics of cell division and cell expansion. The position of the leaf systematically affected the length of the division zone, which accounted for most of differences in length of the growth zone. Leaf position also influenced, but not systematically, *LER* and the relative rate of cell expansion and division. Two position-independent characteristics were found on all genotypes: the length of cells in the basal part of the division zone, and the number of cell-group-specific doubling period of extension after last division. Only the later varied with genotype, and was responsible for the genotypic variability of mature cell length.

P0024. Alfalfa Mob1-like genes are involved in cell proliferation and plant reproduction

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Mps-one-binder (Mob) proteins play an important role in yeast cell division. We cloned two *Mob1*-like genes from *Medicago sativa* L. and showed that one gene (*MsMob1-1*) is constitutively expressed while the other (*MsMob1-2*) is expressed only in flower buds during sporogenesis. A polyclonal antibody was raised against *MsMob1* proteins and used for immunolocalization in dividing and non-dividing cells. The results show that the *Mob1*-like proteins are regulated in a cell cycle-dependent manner during cell proliferation. Cytoplasmic localization was faint and diffuse during G₁ and S, and become pronounced and concentrated into punctuate and fibrillar structures in G₂. During cytokinesis, the protein marked the progressive formation of the septum. *In situ* mRNA localization as well as protein immunolocalization proved that *MsMob1*-like genes are also expressed in degenerating megasporangia of normal ovules and in enlarged MMCs and embryo sacs of apomeiotic ovules. Gene products were also found in tapetal cells of anthers undergoing programmed cell death. These results suggest that *MsMob1*-like genes are involved in cell proliferation and in gametogenesis.

P0025. Nocturnal growth rhythm in leaves of Populus deltoides is controlled by cell proliferation.

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Expanding leaves show marked diel growth patterns. As a first step toward identifying the genes and processes controlling temporal variations in leaf growth, we compared the transcriptomes of *Populus deltoides* leaves at times with well-defined growth rates. Rapidly expanding and fully expanded leaves were harvested at times of high and low growth rates during the night as quantified by an online growth-monitoring system based on the image sequence analysis method. In contrast to previously analysed plants with a pronounced base-tip gradient, *P. deltoides*, which lacks a base-tip gradient across the lamina, showed maximal and minimal growth at dusk and dawn, respectively. Comparison of the transcript profiles highlighted a concerted regulation of ribosomal protein genes during nocturnal temporal growth. Ribosome biosynthesis, a regulatory process in cell cycle, appears to respond to carbon flux from starch breakdown during the night, resulting in growth deceleration at dawn. We discuss the development of distinct spatio-temporal leaf growth patterns in connection with cell division and cell expansion.

P0026. The role of ethylene in the timing of developmental transitions

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The regulation of the timing of developmental events is crucial for a plant's survival given the environmental conditions. The gene, *FUS3*, has been identified in *Arabidopsis* as a key regulator of the transition from the embryonic to vegetative stage of development. A novel role for *FUS3* in the timing of post-embryonic events has also been discovered from expression and functional analyses of the loss-of-function *fus3* mutant. To investigate the function of *FUS3* during these stages, we have used an artificial *FUS3* induction system by fusing *FUS3* to a glucocorticoid receptor. By inducing *FUS3* activity during shoot development, we have created a system that retards the transition from juvenile to adult programs. Using a genome wide approach, genes involved in ethylene synthesis and signaling that display a different transcription profile over time, were identified. The leaf profile of an ethylene signaling mutant, *ein2*, and the use of morphological markers reveal that indeed *ein2* plants do not undergo the transition from juvenile to adult. These results posit a novel role for ethylene as a positive regulator of phase transition in the shoot.

P0027. ENOD40 affects cell size in non legume species

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ENOD40 gene expression is associated with several developmental processes, but its function is still unknown. In an effort to elucidate the function of this gene, *ENOD40* gene was introduced in the genome of *Arabidopsis thaliana* and tobacco BY-2 cells. This approach allowed us to monitor the effect of over-expression of *ENOD40* both at the organism and cellular level. *Arabidopsis* plants over-expressing *ENOD40* did not show remarkable growth abnormalities, but some tissues revealed significantly smaller cells. *Arabidopsis* protoplasts transiently expressing the gene showed reduced expansion, and the same result was obtained with the administration of the peptide. BY-2 cells steadily over-expressing *ENOD40* were impaired in elongation growth, which is consistent with the observations in *Arabidopsis*.

Thus *ENOD40* most likely is involved in a process that regulates cell growth. Analyses of the components involved herein will be discussed.

P0028. On the regulation mechanisms for the direction of division planes upon heterophyllous leaf formation in Ludwigia arcuata (Onagraceae)

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A heterophyllous plant of *Ludwigia arcuata* changes the leaf form upon environmental changes. When a shoot of *L. arcuata* was

transferred from terrestrial condition to submergence, newly developed leaves became narrow submerged type leaves, while old leaves were still round terrestrial ones. Basal region of young developing leaves fully responded to submergence, resulting in the formation of spoon-shaped leaves. Such changes of leaf shapes were ascribed to change in numbers of epidermal cells in the transverse direction. When we observed the orientation of cell divisions in such developing leaves, directions of cell divisions were classified into two directions: one was parallel to the leaf long axis, while the other was transverse to the leaf long axis. Cell divisions for the transverse direction contributed to the increase of leaf width. Ethylene treatment that induced the formation of narrow leaves decreased the activity of cell divisions in the transverse direction. As these observations suggest the presence of a mechanism that regulates the direction of cell division in leaf development; we propose a novel mechanism for leaf development.

P0029. Isolation of Soybean Seed Coat Peroxidase By Ion Exchange HPLC

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Peroxidases(1.11.1.7) are Heme-containing proteins, widely distributed in nature, where play a role in a variety of physiological process such as the structural integrity of cell walls. Peroxidases are also associated with plant defence responses and resistance to pathogens.

Soybean hull peroxidase(SBP), an anionic glycoprotein with isoelectric point 4.1 and M.W. 37 Kda, is found in substantial amounts in soybean hulls, a byproduct of the soybean industry. It exists essentially as single isozyme in hull. It has usually high thermal stability even at low pH.

Commercial preparations such as sigma have low activity and contains several contaminants, so we were interested in developing a method to quickly purify SBP. Fast detection of SBP is also useful in physiological studies.

We purified SBP, by using Anion Exchange High Performance Liquid Chromatography(AEHPLC).

As the quantity of peroxidase is important in plant physiological studies, HPLC provide a more reliable method.

In this work we can achieved purified peroxidase with RZ 2.0.

P0030. Triple Test Cross analysis for genetic components of salinity tolerance in spring wheat

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Soil salinity poses considerable problems for agriculture and receives much attention from plant breeders. The identification of genes whose expression enables plants to adapt and/or tolerate salt stress is essential for breeding programs but little is known about the genetic mechanisms of traits in saline conditions.

Data obtained from 75 families produced by crossing 25 F₂ plants derived from a cross between two spring hexaploid wheats, Siete Cerros (salt tolerant) and Axona (salt sensitive) to their parents and their F₁ progenies, was subjected to triple test cross analysis. Genetic components (epistasis, additive & dominance) and their interactions with the environment (control - salinity) were detected for heading date, days to maturity, final plant height, spike length, ear weight, straw weight, number of grains per ear, grain yield per plant, 1000 grain weight, whole plant weight and harvest index. Epistasis was presented only for days to maturity ('j' and 'l' types) and plant height ('i' type) at control and spike length ('j' and 'l' types) at salinity condition. Additive component (D) was more important than dominance (H) especially in salinity condition. Dominance ratio, (H/D)^{1/2}, was less than unity in both environments and heritability (h^2) decreased for all traits at salinity condition.

P0031. Characterisation of calcium-dependent protein kinase (CDPK) isoforms of *Arabidopsis thaliana* in stress responses

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Plants are constantly exposed to changes in their environment and perceive a variety of biotic and abiotic stresses such as pathogen

attack, cold or drought. Among the earliest cellular responses to such stress stimuli are changes in the cytoplasmic calcium concentrations. Calcium-dependent protein kinases (CDPKs) may function as potential sensors that decode the elevation of calcium concentration into enhanced protein kinase activity and subsequent downstream signalling events. *Arabidopsis thaliana* contains 34 CDPK isoforms. Evidence has been provided for CDPKs to participate in environmental stress signalling and CDPK transcript elevation was reported after exposure of *Arabidopsis thaliana* to cold, salt and drought. We have characterized the biological function of selected *Arabidopsis thaliana* CDPKs in specific signalling pathways by combining biochemical and reverse genetic approaches.

P0032. Influence of extracts of some pharmacological active plants to the seeds germinations of weeds invasive plants

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Influence of one plant to another through their natural metabolites (from different plant organs or different products of dissolution of non-lived plants) is named as allelopathy. In this study were analysing influences of water extracts of pharmacological active plants (*Alnus glutinosa*, *Thymus serpyllum* and *Tagetes minima*) to seeds of weeds of invasive plants (*Amaranthus retroflexus*, *Setaria glauca*, *Echinochloa crus-galli*, *Galinsoga parviflora*). Comparative investigation of influence of water extracts (in concentration of 0,1 and 0,5%) to seeds germination, of invasive weed plants, showed that all extracts with their pharmacological active compound had effects on seed germination, except to the species *Galinsoga parviflora*. The pressure of extracts showed the best results for the treated species *Amaranthus retroflexus*, with decreasing of germination in 50 %. Statistical differences were significant in the number of germinated seeds between non treated and treated plants with different concentration of extracts.

P0033. Metabolic response of the cyanobacterium *Anabaena variabilis* to iron limitation

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Under conditions of iron stress, the many bacteria and some cyanobacteria and algae secrete organic compounds, called siderophores that specifically bind and solubilize Fe.

This contribution deals with the isolation and identification of siderophore-like substances in culture of cyanobacterium, *Anabaena variabilis* under low iron conditions.

The iron-binding ligands were isolated by extraction and liquid column chromatography. The siderophore-like substances were analyzed in the examined samples by the chemical assays: CAS AD and the Arnow test. Biological activity of extracts was determined using specific bioassays with the apply of mutated bacterial strains (*Microbacterium flavescens* JG-9 and *Morganella morganii* SBK-3).

The extracts examined showed strongly positive responses. Detectable levels of hydroxamate and -hydroxy / -ketohydroxy acids type siderophores were produced by *Anabaena variabilis*.

The study was carried out as part of statutory programme of the Institute of Oceanology in Sopot (No.II.3.1) and was supported by the Polish State Committee of Scientific Research (grant No.3P04F 059 23, 2002-2005).

P0034. Salt and drought induced alterations in proteins and some enzymes activities in seedlings and calli of fenugreek (*Trigonella foenum-graecum* L.)

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Fenugreek is an annual legume and important pharmaceutical plant. It is distributed in Africa, Europe, and Asia. Fenugreek grows in non-saline soils, but also it can be grown in relatively saline soils. Besides, it can not tolerate drought stress. In this research we investigated the effects of NaCl (0-200 mM) and drought stress (PEG 4000, PEG 6000 & Mannitol 0-20%) on the germination percentage, content of proline and proteins, and activity of antioxidant enzymes in seedlings and calli. Protein and proline content was determined by the spectrophotometric method. SDS-PAGE system was used for qualitative changes of proteins. According to obtained results seedlings tolerate NaCl up to 100 mM and PEG and mannitol up to 10 %. Proteins and proline content increased under salt and drought stress. Moreover,

alterations of some antioxidant enzymes such as peroxidase, catalase and polyphenol oxidase in seedlings and calli were studied under stress using spectrophotometric method and PAGE system.

P0035. Uptake and accumulation of aluminium in root cells of *Arabidopsis thaliana*

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Aluminium toxicity is an important growth-limiting factor in acid soils; experiments have shown that the root apex is the primary site of aluminium-induced injury in plants. The main symptom of aluminium effect is a decrease of root growth; first physiological responses are observed shortly after aluminium application.

Root growth of *Arabidopsis* plants cultivated for 7 days on aluminium-containing medium (10-300 µM) was inhibited in a concentration-dependent manner. We documented changes in the cytoarchitecture and behaviour of exo-/endocytotic vesicles in root hairs as an immediate response to aluminium. For localisation and uptake of aluminium in root cells we used specific fluorescence dyes: morin for detection of aluminium and FM4-64 for labelling of the plasma membrane. Results showed that aluminium was excluded to the apoplast in elongated and mature cells, whereas it was taken into the cytoplasm of cells in meristematic and transition zones within 1,5 hours. 4 hours after treatment aluminium had further progressed and concentrated into provacuolar compartments and vacuoles.

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P0036. Phosphatidylcholin-specific phospholipase C may play a role in aluminum toxicity in plants

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Aluminum (Al) is a highly cytotoxic metal for plants, but the molecular base of Al toxicity is still far from being understood. The most important physiological consequence of Al toxicity is changes in root morphology suggesting a role of root cytoskeleton as a target structure. The important role of phospholipid degrading enzymes, phospholipases C (PLC) and D in regulation of cytoskeleton remodelling is now evident. The aim of our work is to study molecular mechanism of aluminum toxicity with special respect to the role of plant phospholipases.

Fluorescently labelled phosphatidylcholine was used to study changes of phospholipid profile after aluminum treatment of tobacco cell culture BY2. Products of phospholipases action were analysed by HP-TLC. The amount of diacylglycerol (product of PC-PLC) decreased within 30 min of Al treatment. Later on, PC-PLC activity *in vitro* was detected in the same experimental material and basic biochemical properties of the PC-PLC were determined. Presented results suggest a role of the PC-PLC in molecular mechanism of aluminum toxicity.

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P0037. Functional Characterization of a Rice Choline Kinase Gene in Transgenic Plant

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The rice cDNA clone that encodes osCK1 (*Oryza sativa* choline kinase 1) was isolated by differential screening from cold treated rice cDNA library. While phosphatidylcholine (PC) has known containing a major role in plant stress adaptation, the choline kinase, the first enzyme of CDP-choline pathway for PC biosynthesis, may contain a possible regulatory role in stressed plants. The expression level of osCK1 transcripts specifically increased in cold treatment but not changed in ABA or salt treatments in rice. The osCK1 gene was constitutively over-expressed in rice, and microarray analysis of transgenic plants revealed that over-expression of osCK1 enhanced induction of several genes supposed to be involved in calcium signaling pathways. In Northern blot analysis, genes containing calmodulin binding motif or unknown function were up-regulated in the

transgenic rice. These results hypothesize that over-expression of choline kinase may activate the gene expression involving in calcium signaling pathways. The molecular function of osCK1 responding to biotic and abiotic stress in transgenic rice will be further discussed in this presentation.

P0038. Floral Ontogeny of Soybean (*Glycine max* (L.) Merrill) under Drought Stress

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The effects of drought stress on floral ontogeny were examined in soybean (*Glycine max* (L.) Merrill) cv. SJ5. The drought treatment was performed by application of 30% of plant-available water to V4-stage soybeans, which consisted of 4 internodes, and floral meristem was initiated at every axillary bud. The drought treatment was ended when the first flower bloomed (R1 stage). The stressed plant showed the significant reduction in height, fresh weight and dry weight when compared to the non-stress controls. Drought stress treatment applied during V4-R1 stage of soybean reduced the number of flowers by 63%. Moreover, it also affected the pollen fertility. The *in vitro* pollen germination test, performed with the pollens from blooming flowers, showed that the stress decreased germination percentage by 82%. However, drought stress had no effect on stigma receptivity. Therefore, at the reproductive stage, the severe effect of drought stress on soybean productivity was caused by both the floral development prohibition and reduction in pollen viability.

P0039. Expression Analysis of GSK-3/SHAGGY-Like Kinases in *Arabidopsis thaliana*

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Glycogen Synthase Kinase-3/SHAGGY-like kinases (GSKs) are evolutionary highly conserved, multifunctional non-receptor Serine/Threonine protein kinases.

In *Arabidopsis thaliana* there are ten different GSKs (ASKs) encoded by a multigene family. The ASKs can be divided into four groups based on their sequence similarity. So far, studies have implicated their involvement in flower development, brassinosteroid signalling, and stress adaptation responses.

To clarify the functions of each ASK in cell signal transduction, we study the temporal and tissue specific expression patterns. Therefore, the distinct ASK promoters were fused to the GUS reporter gene. Expression patterns are analysed under defined growth conditions during plant development, hormone treatment, and in response to biotic/abiotic stresses, including pathogen infection, leaf wounding, hyperosmotic stress, extreme temperature as well as different light conditions.

P0040. Effects of Exogenous Abscisic Acid on Salt Tolerance and Proline Accumulation of Rice (*Oryza sativa* L.) Seedlings

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The effects of abscisic acid (ABA) on salt tolerance and proline accumulation were studied in the two similar genetic background rice lines, which have the different salt-tolerant ability. When the two-week old, salt-sensitive seedlings (LPT123) were grown in salt-stress condition by addition of 0.5% NaCl in nutrient solution, it was resulted in shoot fresh weight loss and the stable root growth during four weeks of the stress period. Contrastly, the salt-tolerant rice seedlings (LPT123-TC171) grown in the same condition, showed the maintenance of shoot fresh weight and root dry weight accumulation. The application of 100 µM ABA everyday on leaf blade reduced salt-stress effects in both rice lines by showing the better growth and survival rate. ABA application significantly induced early proline accumulation in both rice lines via the up-regulation of *OsP5CS* gene expression. Our results suggest that the early accumulation of proline, induced by exogenous ABA may be responsible for the increase in salt tolerant ability in rice seedlings.

P0041. Temperature stress tolerance and heat shock granule complexes in *Lupinus albus*

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Living organisms respond to high temperatures by repressing the synthesis of most proteins and by inducing the synthesis of a small group of proteins—the heat shock proteins (HSP). One of the physiological functions attributed to the HSP is their involvement in the thermotolerance acquisition. Despite the suggested role of HSP in thermotolerance, the individual function and identity of most HSP remains unknown, particularly in the case of plants. The HSP20 family shows some characteristics that differ from the other HSPs. It has been suggested that the physiological function of HSP20 family is related with the cytosolic formation of heat shock granules (HSG). The abundance of HSG under stress conditions namely, high temperature, amounting to 1% of total cell protein suggests their functional importance in survival at high temperatures. Therefore, the aim of the present study was to analyse those aspects that are known to be directly involved in the acquisition of thermotolerance, namely the HSP20 family and the heat shock granules (HSG) in *Lupinus albus*.

P0042. Close association of an FtsH protease with photosystem II in higher plants

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In chloroplasts, there are several protease families, including Clp, DegP and FtsH. Recently an FtsH protease (FtsH2) was suggested to be involved in the degradation of photodamaged D1 protein under light stress. In this study, we carried out Western blot analysis with spinach photosystem II membranes and core complexes prepared by the treatment of the photosystem II membranes by HTG (n-heptyl thioglucoside) using antibodies against the FtsH proteases (DS9 from tobacco and VAR2 from *Arabidopsis*), and showed that the FtsH protease is present in the vicinity of photosystem II. With a chemical cross-linker bis(sulfosuccinimidyl)suberate sodium salt, we showed the FtsH2 and the D1 protein to form a large cross-linked products by the illumination of the photosystem II membranes. These results suggest that the photodamaged D1 protein is recognized as a substrate by the FtsH protease. Our data also suggest the possibility that D1 degradation takes place in the stacked region of thylakoids.

P0043. Degradation of the D1 protein of Photosystem II in *Synechocystis* PCC6803 under heat stress - the role of an FtsH protease

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With wild type and FtsH (slr0228) mutant cells of *Synechocystis* PCC6803, cell growth, oxygen-evolution and degradation of the D1 protein of PS II were examined under heat stress. Wild type cells grew normally under heat stress (40°C) conditions, while FtsH cells were sensitive to the heat stress and showed bleaching of pigments. Oxygen evolving activity of wild type cells showed no decrease upon heat stress (42°C) unless lincomycin was added. Oxygen evolution of the mutant cells suffered from the heat stress in the presence or absence of lincomycin. Western blot analysis with the antibody against the D1 protein showed that the amount of the D1 protein decreased upon heat treatment of the wild type cells. On the other hand, no apparent loss of the D1 protein was observed with the mutant cells under the same stress conditions. These results suggest that the FtsH protease is involved in the degradation of the D1 protein under heat stress conditions.

P0044. Cleavage of the D1 protein of spinach PS II under heat stress: an FtsH protease is responsible for the proteolysis

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When spinach thylakoids were treated with heat (40°C for 30 min), the reaction center-binding D1 protein of PS II was cleaved and a 23 kDa N-terminal fragment was produced. The proteolysis was specific to the D1 protein and no other proteins around the D1 protein were degraded. The cleavage of the D1 protein was stimulated by the addition of Zn and inhibited by EDTA, indicating participation of a metallo-protease. The protease was solubilized when the thylakoids were treated with 2M KSCN. The protease activity in the supernatant of the KSCN-treated thylakoids was

detected by gelatin-activity gel electrophoresis and size of the protease was about 70 kDa. Western blot analysis using antibody against FtsH proteases (anti-DS9 and anti-VAR2) showed that the supernatant contains an FtsH protease. Reconstitution of the FtsH-containing supernatant fraction with the thylakoids was also successfully carried out. Our results strongly suggest that the FtsH protease is responsible for the proteolysis of the D1 protein under heat stress.

P0045. Adaptive responses to high salinity of two subspecies of *Aster tripolium* on different nitrogen sources

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Soil salinity is an important agricultural problem. One possible way to use affected fields is planting salt tolerant crops such as *Aster tripolium* L.

Aster tripolium is a typical halophyte species with two horizontally isolated subspecies. The ssp. *tripolium* is a maritime halophyte, while ssp. *pannonicus* is common on the continental alkaline salty meadows.

Adaptive responses to high salinity of two subspecies of *Aster tripolium* were studied on different pH values and Na⁺ concentrations and on different nitrogen sources. One of our interesting result was that young leaves of *Aster tripolium* ssp. *tripolium* treated with ammonium and relatively low NaCl concentrations became chlorotic, while on high salt concentrations showed the signs of ammonium toxicity. Ion concentrations (Na, Fe, K, Ca and Mg), nitrate reductase (NR) and glutamine synthetase (GS) activity were measured in both leaves and roots. We conclude that ion concentrations, NR and GS activities have changed differentially in the two subspecies, which can be regarded as adaptive responses to their environmental conditions.

P0046. Comparison of wheat, barley and rye with respect to salt tolerance at the germination stage

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Soil salinity is one of the most important environmental factors, which limit the crop production in many parts of the world, particularly in areas with arid and semi-arid climatic conditions. Mismanagement of the valuable resource water enhances the desertification of agricultural arable land by secondary salinisation. Salt tolerant crops could be used to reclaim salt affected soils or for the improvement of the yield.

In 2003 a programme was initiated at the Genebank of Gatersleben for the screening of salt tolerance in cereals. 569 hexaploid and 125 tetraploid wheat and 108 barley accessions of the genebank collection were tested at the germination stage. In addition several rye accessions and inbred lines from the breeding programme of the Martin-Luther-University Halle-Wittenberg were investigated. Germination tests were carried out on filter paper in plastic boxes by using three different concentrations of NaCl solutions (1.5%, 2.0% and 2.5%) and distilled water as control. After ten days in a climatic chamber with a light and dark photoperiod of 12 hours and a constant temperature of 20°C, the lines were scored according to the scheme of Mano et al. (1996).

P0047. Effects of zinc and copper on expression of metallothionein genes, and on polyamine content in *in vitro* micropropagated *Populus alba* L.

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In an effort to acquire knowledge of the basic processes underlying metal detoxification and tolerance in poplar, we isolated *Populus nigra* metallothionein (MT) genes on the basis of *P. trichocarpa* cDNA sequences available on DOE JOINT GENOME INSTITUTE databases. Three genes, belonging to class 1, 2 and 3, were cloned and sequenced. Their expression patterns were northern blot analysed in *in vitro* micropropagated shoots of the *P. alba* clone "Villafranca" after exposure for 1, 3, 6 and 15 days to ZnSO₄ and CuCl₂. Results show that a) Zn (> 1 mM) and Cu (> 0.1 mM) induced leaf chlorosis; b) rooting of shoots was delayed and reduced (Zn >1 mM), or totally inhibited (Cu); c) transcript levels of

MT1, MT2 and MT3 genes were enhanced in a dose-dependent manner in the leaves of shoots treated with Zn, whereas Cu did not stimulate expression of MT genes. Since polyamines (PAs) are known to be involved in various abiotic stress responses, free and conjugated leaf PA content was monitored during treatment with Zn or Cu, and was found to correlate positively with metal concentration.

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Author 1, 2 and 3 contributed equally to the present work.

P0048. Molecular genetic study on the abiotic stress signal transduction in rice (*Oriza sativa* L.)

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Although a part of signal transduction of abiotic stress response has been elucidated in dicot plants, it remains unclear in monocot plants. Using a cold-sensitive rice plant as a model, the stress response mechanism has been studied. First, drought and cold responsive *OsDhn1* and *OsAsr1* were identified from the seed coat ESTs. Both genes were induced by cold stress in the reproductive organ, preferentially in the lower epidermis of the palea and lemma. Second, *OsAsr1* and *OsDhn1* were up-regulated in the transgenic rice expressing *CBF1/DREB1b*, suggesting the possible regulation of those genes by the CBF/DREB stress-signaling pathway. Third, among nine genes retrieved from rice genome database *OsDREB1E*, *OsDREB1F*, *OsDREB1G*, *OsDREB1H*, and *OsDREB1I* were novel. While *OsDREB1E* and *OsDREB1F* were induced by cold, *OsDREB1G* and *OsDREB1H* by drought, *OsDREB1I* was induced by both cold and drought. Fourth, five rice *ICE1* homologues of bHLH type genes were retrieved from the rice genomic database and their characterization is being conducted. Finally, Transactivation assay revealed that *OsDREB1A* and *CBF1*, but not *OsDREB1I*, could induce *OsDhn1* in the rice protoplasts.

P0049. The role of glutathione S-transferases in the drought stress tolerance of different wheat genotypes

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Investigation of enzymatic antioxidant activities of *Triticum aestivum* varieties with different drought tolerance under osmotic stress revealed the importance of glutathione S-transferases (GSTs). GSTs are ubiquitous enzymes catalysing the addition of reduced glutathione to cytotoxic substrates thus protecting against environmental stress by detoxifying reactive products. The more tolerant wheat lines had higher GST activities in their roots, and enzyme activities increased differentially by 400 mOsm polyethylene glycol (PEG 6000) treatment. Our aim was to identify the water stress-related GST genes in wheat lines and compare the expression pattern of these genes in different wheat varieties under osmotic stress.

Searching for drought tolerance related genes revealed that expression of GST genes elevated under water stress (Györgyey et al., unpublished results). Phylogenetic analysis of wheat GSTs was performed *in silico* using TIGR database and Clustal software. Homology was found between the upregulated sequences and the GST coding TCs in the GenBank database; different GST genes from GST classes Phi and Zeta were chosen for further estimation.

P0050. Biochemical expression on salt-tolerant and salt-sensitive lines of rice (*Oryza sativa* L.) responses to salt stress

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The aim of this investigation is to express on betaine aldehyde dehydrogenase (BADH) for glycinebetaine (Glybet) accumulation in *in-vitro* rice seedlings using salt stress conditions. In both salt-tolerant and salt-sensitive lines, the BADH enzyme progressively increased during the first few hours after NaCl exposure until reach to the maximum point after 72 h and then dropped. It was clearly showed that the seedlings of salt-tolerant line had the highest level of BADH enzyme at 72 h about 2.6 times more than those of salt-

sensitive line. Moreover, the highest amount of Glybet was detected in salt-tolerant line that was 1.2 times more than in salt-sensitive line at 96 h. The BADH activities were positively related to Glybet accumulation in salt-tolerant and salt-sensitive lines. It should be noted that the expression of BADH enzyme in salt-tolerant line cultured under salt stress exhibited a significant increase in Glybet biosynthesis and accumulation higher than salt-sensitive line. The accumulation of Glybet immediately following the initial exposure of rice to salt stress may function as a defensive response to salt stress conditions.

P0051. Proline produced due to NaCl pre-exposure is the prime cause behind reduced sensitivity of *Azolla pinnata* plants to UV-B radiation

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Sensitivity of *Azolla pinnata* plants to NaCl (40 and 60 mM) for 8 days and daily UV-B exposure of 3 and 6 h corresponding to 4.32 and 6.48 KJm⁻² till 8 days was investigated. NaCl and UV-B resulted in visible injury symptoms. At 40 mM NaCl treatment and 3 h UV-B exposure injury symptoms in the form of chlorosis and marginal necrosis was observed with a corresponding increase in peroxidase activity. However, exposure to 60 mM NaCl and 6 h UV-B resulted in chlorosis followed by necrosis and nearly 2.5 fold increase in peroxidase activity. Growth, chlorophyll content and PS II activity decreased due to NaCl but increased proline, H₂O₂, electrolyte leakage and lipid peroxidation. Similar results were obtained following UV-B exposure. Simultaneous exposure to UV-B and NaCl produced an additive effect. However, pre-exposure to NaCl decreased H₂O₂ generation, lipid peroxidation and electrolyte leakage considerably. These results have been confirmed when plants were exogenously supplied with proline (1-5 mM) and subsequently exposed to UV-B. The role of proline accumulated under salt conditions enhanced resistance of *Azolla* plants to UV-B exposure.

P0052. Characterisation of GSK-3/Shaggy-like Kinases in *Arabidopsis thaliana*

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The ten ASK proteins (*Arabidopsis thaliana* GSK-3/Shaggy-like Kinases) are homologues of the mammalian GSK-3 (Glycogen Synthase Kinase 3) and the *Drosophila* melanogaster Shaggy kinases. They can be grouped into four classes, according to their sequence similarity. Plant GSK-3/ Shaggy-like kinases have been shown to be involved in many different signal transduction processes, ranging from flower development and hormone perception to stress responses. To characterise the *Arabidopsis* members of this kinase family, we have generated transgenic plants overexpressing epitope-tagged versions of the kinases. Furthermore, we have acquired the respective T-DNA insertion lines from SALK, Sail and GABI-Kat and analysed them by PCR and Southern blotting. Phenotypic characterisation of overexpression and knockout plants are in progress. Preliminary transient expression assays in protoplasts revealed highly different protein levels and kinase activities, even between closely related ASKs, indicating involvement in different biological processes.

P0053. Acclimation of poplar trees to heavy metals in polluted habitats: II. Alcoholic fermentation in fine roots of *Populus nigra* and *Populus deltoides*

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We studied effects of chronic industrial pollution on the metabolic activity of roots of *Populus nigra* L. and *P. deltoides* Bartr. ex Marsh. The trees had grown for 20-30 years near a copper smelter - at Glogów (51°40'N, 16°05'E, buffer zone A) and at Bogomice (51°41'N, 16°01'E, buffer zone B) - and in a control area at Kórnik (52°15'N, 17°04'E). We assessed the 'vitality' of fine roots, mycorrhizae, carbohydrates, activity of pyruvate decarboxylase, fermentative enzymes: alcohol dehydrogenase and lactate dehydrogenase. Also microbial activity and chemical properties of the soil were analysed. Our results attest to seasonal and interspecific differences in all the parameters. Generally, the microbial activity of the soil and mycorrhizal colonization of fine

roots sharply diminished in zone B and disappeared in zone A. In contrast, the 'vitality' of fine roots decreased more strongly in zone B than in zone A. In the area affected by the copper smelter, starch, sucrose, glucose and fructose contents of fine roots and activities of fermentative enzymes increased in *P. nigra* and were reduced or unchanged in *P. deltoides*.

P0054. Etio-chloroplasts to nucleus signalling and Lhc gene expression in *Pinus sylvestris* cotyledons

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It is known that in angiosperms the photodependent expression of Lhc nuclear genes (photo-genes) is also regulated through an inhibitory signal produced by photodamaged chloroplasts as consequence of chlorophyll precursors accumulation. No information is available about gymnosperms where Lhc gene expression is usually not induced but only stimulated by light.

We studied the consequences of an inhibited carotenoid synthesis induced by herbicides on cotyledonal etio-chloroplasts of dark-grown *Pinus sylvestris* plantlets. The treatment had negative effects (not due to photodamage) on ultrastructural organization, chlorophyll content and molecular composition of thylakoid membranes. An interesting finding was the production by the altered etio-chloroplasts of a signal inhibiting the expression of nuclear Lhc genes, which in this species are constitutively transcribed in darkness. The inhibition was correlated with an increase of soluble chlorophyll precursors. Thus, the plastidic signal which modulates nuclear Lhc gene transcription already exists in gymnosperms and its chemical nature seems to be the same of angiosperms.

P0055. Stress proteins and phytohormones: their role in formation of plant resistance.

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Using the disc-electrophoresis methods, we have studied protein biosynthesis of different plants, including 11 species of *Orchidaceae*, some other tropical and subtropical plants, 9 different fruit plants, and 4 cultivars of *Triticum aestivum* L. under stresses factors such as high and low temperature, clinostating, radioactive irradiation and osmotic shock. Specific and unspecific reactions of plants protein system on stresses were found. *De novo* synthesis of 35 and 45 kD polypeptides were observed in total and mitochondrial proteins fractions after heat-shock and radioactive irradiation. This suggests that mitochondria participate in formation of plant resistance.

Intensive synthesis of ABA revealed as the universal reaction of all studied plants on action of different kinds of stresses. Specific changes in balance of phytohormones were found under different stresses. We observed the correlation between endogenous ABA, IAA and cytokinin level and plant resistance. We also found the interaction between the process of biosynthesis of proteins and phytohormone balance, as well as their direct participation in formation of plant resistance.

P0056. Phytohormonal complex of *Syngonium* under the conditions of hermetically sealed vessel

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Plants growing in especial hermetic vessel are of great interest so as it makes it possible to simulate one of the space flight factors - oxygen deficit, to establish plants with tolerance to such conditions for the following employment at the orbital stations. We didn't determine essential morphological changes in *Syngonium* plants after nine months of staying in hermetically sealed vessel comparing with plants growing in open soil. It is probably the result of these plants high tolerance to hypoxia. To reveal this adaptation mechanism the content of endogenous phytohormones (IAA, ABA, cytokinins and gibberellins) has been investigated in *Syngonium* roots and shoots by HPLC. Studies on the hormonal balance *Syngonium* in this conditions were conducted for the first time. It was determined that all phytohormones level was essential decreased in the experimental plants comparing with the control ones. The obtained results has been analysed as regards the relation of adaptive properties of *Syngonium* plants and hormonal system lability.

P0057. The interaction between WRKY53, a senescence specific transcription factor, and P9, a jasmonic acid inducible protein conferring resistance to bacterial and fungal links senescence to the pathogen responsive pathway

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Using yeast two hybrid system, we found that WRKY53 interacted with a putative jasmonic acid inducible protein (p9), WRKY53 and p9 colocalized in nucleus. The results of RNA blot showed that p9 was induced in col-0 treated with JA, p9 expression was completely block by jar1-1 but decreased in npr1-1 and col-0 treated with SA. Conversely, WRKY53 expression was suppressed in col-0 and jar1-1 treated with JA, but increased in npr1-1 and col-0 treated with SA. The infection result of bacterial and fungal showed that the 35S:p9 significantly decrease the growth of bacterial and damage degree of fungal in leaf region, but p9 ko-line increase the growth of the bacterial and damage degree of fungal. However, There is no significant difference in 35S:WRKY53 line and WRKY53 ko-line. Moreover, 35S:WRKY53 suppressed the expression of p9 in RNA level and WRKY53 ko-line increased p9 expression. We suggest that WRKY53, as a senescence specific factor, act upstream of NPR1 in a SA-dependent signal pathway, act as an activator of SA-induced genes and a repressor of JA-responsive genes, through interacting with p9 integrating senescence signals into pathogen responsive pathway.

P0058. Hormonal complex of two different ecotypes of *Sium latifolium* L.

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Sium latifolium L. plants can grow in different water conditions: dipping into water (up to 50% of plant body) and on land near the coast. These two plant ecotypes differ only in their sizes - 1.5-2 m and 25 cm correspondently. To understand the physiological mechanisms of such phenotypic plasticity the content of endogenous phytohormones in vegetative and reproductive organs of *S. latifolium* plants, growing in different ecological conditions (in water and on land) was studied during vegetation by HPLC method. It was shown that ABA content was essential higher and cytokinin and IAA level was lower in land plants comparing with water ones. Differences in free and bound phytohormones content were constant during ontogenesis. It is possible that *S. latifolium* plants capacity to form different phenotypes within the same genotype is connected with the ability to maintain the phytohormonal balance in conformity to the environment during long time.

P0059. Intracellular localization of GABA and free amino acids in plants under hypoxic stress and high concentration carbon dioxide

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Under hypoxia in plants the exchange processes of free amino acids get disturbed. The effect of hypoxia and CO₂-media (6-24h) on distribution of free amino acids between vacuolar and cytoplasm compartment in leaves of seedlings with different tolerance was studied under usage of DMSO.

It was observed that in all plants under hypoxia the most changes occurred in GABA and alanine (Ala) content. In midtolerant seedlings (corn, soybean) the GABA level increased 3-7 fold and in intolerant (wheat, pea) 1.5-2.5 fold. Accumulation of GABA in intolerant plants occurred in vacuole and in midtolerant plants in vacuole and cytoplasm. The level of Ala increased in 30-150% under hypoxia in intolerant plant cells in cytoplasm, but in midtolerant in vacuolar one. The content of glutamate in all plants under hypoxia didn't change. The level of aspartate in leguminous was decreased on 20-40% in vacuole. Under 24h of hypoxia the increase of vacuolar compartment of GABA and Ala was observed. CO₂-media stimulated more substantial changes in amino acids than hypoxia.

The important role of vacuole in accumulation of stress amino acids GABA and Ala under hypoxic stress was proved.

P0060. Field trial validation of a terminal drought tolerance QTL in pearl millet

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Several putative QTLs were mapped in pearl millet [*Pennisetum glaucum* (L.) R. Br.] for grain and stover yield components *per se* and yield maintenance under terminal drought stress. Marker-assisted selection (MAS) for these QTLs facilitated the development of near isogenic lines (NILs). Nineteen homozygous NILs and their two parents (donor PRLT 2/89-33 and recurrent H 77/833-2) were testcrossed to five closely related male-sterile testers. The resulting 105 hybrids were evaluated for their agronomic performance in replicated field trials during 2003 and 2004 at Patancheru (rainy & post rainy) and in two drought-prone locations at Rajasthan (rainy). Significant effects of genotype (G), environment (E), and G x E interactions were observed on the grain and stover yield components. Across 14 environments, general combining ability effect of introgression lines ICMR 01029 and ICMR 01031 were substantially better than recurrent parent H 77/833-2 for grain yield. Subsequent line-source irrigation experiment confirmed these findings under moisture gradient and validated the effect of a major QTL for grain yield terminal drought tolerance on LG2 of PRLT 2/89-33.

P0061. Mycorrhizal fungi, modalities for reducing the drought stress during acclimatization of vitroplants-biochemical aspects

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Mycorrhizal fungi established symbiotic association with vascular plant roots. This symbiosis is a bilateral cooperation between partners and supposes structural and ultrastructural changes. Many studies were emphasized that mycorrhizal fungi increase tolerance to extreme conditions, like drought. This tolerance is attributed to changes in the host's photosynthesis process or in levels of carbohydrates and proteins. The transfer of vitroplants to *ex vitro* is one of the most critical factor in the acclimatization and causes often important damage of the biological material. Therefore, we achieve a study regarding the inducing of drought tolerance by experimental colonization of vitroplants during transfer to *ex vitro*. The mycorrhizal colonization induces the reducing of drought stress and decreasing of oxidative processes. The oxidative processes will be monitored by the level of enzyme activity and electrophoretic spectra of oxidoreductases: superoxide-dismutase, catalases and peroxidases. The variation of antioxidant enzymes activity denotes the amplitude of oxidative processes which undergo in the plant cell during acclimatization.

P0062. Hydraulic conductivity of figleaf gourd (*Cucurbita ficifolia*) roots, a species tolerant to low temperature

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Characteristics of low temperature (LT)-tolerant figleaf gourd (*Cucurbita ficifolia* Bouché, FG) root as an ability to absorb water upon exposure to LT was studied. FG roots were able to maintain positive root pressure at LT (8 °C) when measured with root pressure probe. While a sudden introduction of LT lowered the hydraulic conductivity of root (L_p), pre-conditioning of root with LT for 24 h followed by 5 h exposure to 25 °C dramatically increased the L_p. In this case, hydrostatic and osmotic L_p, and cellular hydraulic conductivity (L_p) were increased by a factor of 6.5 and 16.9 and 3.5, respectively. Western blot analysis of the SDS-PAGE confirmed that the aquaporin protein expression in root plasma membrane was marginally increased within 1 d of LT treatment, indicating the increased activity of house-keeping aquaporins that resulted in high L_p and L_p. The activity of proton-ATPase and L_p were insensitive to externally applied hydrogen peroxide. These findings suggest that the aquaporin activity may play an important role upon exposure to LT and that the process of

root acclimation includes the efficiency of aquaporins to transport water across the membrane.

P0063. Early responses of *Chenopodium rubrum* cells to heat shock

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Early cellular responses to heat shock were studied in heterotroph cell suspensions from *Chenopodium rubrum* L. The effect of heat shock was quantified using biochemical parameters related to cellular vitality, membrane function and membrane integrity. It was found that extracellular pH and an alkaline phosphatase activity are more sensitive to heat shock than other parameters like reduction of methylthiazolotetrazolium (MTT) and dichloroindophenol (DCPIP), and cellular release of osmotic content, protein and betalin. Reduction of MTT is ascribed to mitochondrial dehydrogenase activity and reduction of DCPIP to plasma membrane electron transport. Both reductase activities were inhibited by heat shock at 45°C and above. Heat shock at lower temperature had no effect on the reduction of MTT and DCPIP. Heat shock at 30-35°C affected only extracellular pH and an alkaline phosphatase. Comparison of these data indicates that extracellular pH and an alkaline phosphatase respond specifically to heat shock. Thus, changes in extracellular pH and alkaline phosphatase described here are suggested to be involved in signalling of abiotic stress.

P0064. Gene expression detection in single trichome cells of plant leaves by direct RT-PCR amplification of mature mRNAs in response to external stimuli

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A single-cell RT-PCR was conducted to detect gene expression *in situ* in pinpointed trichome cells of tomato leaves. The cytoplasm was removed with the micropipette using a light microscope and directly used for RT-PCR, followed by nested PCR. Two intron-containing genes, glyceraldehydes 3-phosphate dehydrogenase gene and plasma membrane H⁺-ATPase gene were constantly expressed in this tissues and therefore used as the indicator, because of easy detection of shorter-size PCR-products produced by splicing. In addition, the sucking of nucleus-free cellular contents was effective to prevent contamination of genomic DNA led to miss-amplification of corresponding genomic DNA sequences of the intron-less genes in the process of RT-PCR and subsequent nested PCR. Thus, the present technique could be applicable to single trichome cells of tomato leaves for directly detecting their gene expression in response to chemical and physical stimulation.

P0065. The effect of salinity and temperature on germination, growth, development and production of active substances in *Silybum marianum*

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Two types of *S. marianum* (German type and Ahwaz wild type) were studied in the field and laboratory. The laboratory experiments were carried out to determine the effect of seven levels of salinity (0.01, 1, 3, 6, 9, 12 and 15 dS/m) and three levels of temperature (15, 25 and 35 °C) on germination characteristics. The field experiments were carried out to determine salt tolerance of six salinity levels (1, 3, 6, 9, 12 and 15 dS/m) on emergence, growth, development and production of active substances. The results indicated that, salinity affected germination and survival of seedlings at the mentioned temperatures. The optimum temperature for germination of both types was 15 °C. Growth of seedlings was reduced significantly by salinity above 9 dS/m. Other growth parameters were significantly reduced compared to the control plants. Salinity levels had less effects on vegetative growth of Ahwaz wild type. The wild type produced higher silymarin levels in all salinity treatments compared to the German type. The results also indicated that, during the growth, *S. marianum* could survive up to 15 dS/m salinity and produce seeds rich in active substances.

P0066. *flubber (flb)*, a new root hair mutant in *Arabidopsis thaliana*

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Understanding the genetic programme behind the development of a specific cell of an organism will allow us to ultimately manipulate and change the way in which an organism is formed. This project is concerned with understanding the genetic programme that controls root hair formation and development. We carried out a screen and identified a new true breeding mutant in *Arabidopsis thaliana* called *flubber*. The *flubber (flb)* mutant had abnormally short root hairs which showed branching along their length. *flb* plants also had smaller rosette diameters, shorter inflorescence stems, smaller siliques, increased numbers of trichomes and a reduced number of stomata. It is thought that the cell elongation process is affected in *flb* plants. A defect in polarity would account for the branching that is evident along the root hair. Crossing this mutant with other known root hair mutants established that the *FLB* gene acts during the main elongation phase of the root hair development. Mapping of the *FLB* gene is underway and subsequent characterisation of gene products will allow the exact function of *FLB* to be determined.

P0067. Comparative and phylogenetic analyses of the microtubule-associated protein 65 (MAP65) family in plants

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Recently, we have shown that the *Arabidopsis* PLE/MAP65-3 protein binds to microtubules (MTs) and is important for the proper organization of the phragmoplast. Mutated ple alleles fail to execute cytokinesis in a root specific manner. In addition it has been shown that related proteins from yeast, fruitfly, *C. elegans* and human - ASE1, FEO, SPD-1 and PRC1, respectively - have similar functions. PLE/MAP65-3 belongs to a gene family of nine members in *Arabidopsis*. Detailed analyses of the gene and protein structure of all the *Arabidopsis* MAP65s will be presented. Phylogenetic analyses including related proteins of other plants and kingdoms revealed a structuring of the MAP65 family into five subclasses.

Transcript profiles of the nine *Arabidopsis* MAP65 genes were analyzed by real-time RT-PCR at different developmental stages and in diverse plant organs and compared with whole genome microarray data. To functionally characterize the MAP65 family we started a reversed genetic approach and the phenotypes of the identified knock-outs alleles of six members and their double mutants will be presented.

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P0068. Characterization of HYADE, an *Arabidopsis* gene involved in cytokinesis

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Cytokinesis is the final step during cell division. Plant cells, in contrast to animal cells, divide their cytoplasm by constructing a new wall between daughter nuclei. This partition is achieved by phragmoplast guided transport of secretory vesicles carrying cell wall and membrane components towards the plane of cell division. Upon fusion of these vesicles a cell plate is formed which grows centrifugally towards the site at the plasma membrane, that was previously occupied by the preprophase band (PPB).

We have isolated an *Arabidopsis* mutant, *hyade*, that exhibits root specific cytokinesis defects such as irregularly expanded cells, partially formed transverse cell walls and multiple nuclei. During cell division, these multinucleated cells divide synchronously and influence the position of microtubule arrays including PPB, the mitotic spindle and the phragmoplast. Here we present the cloning and molecular characterization of the *HYADE* gene and present evidence for the involvement of the endosomal sorting complexes required for transport (ESCRT) during cytokinesis.

P0069. Dynamics of cytokinin composition in the leaves of winter wheat under high temperature

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Cytokinins is one of the most mobile classes of plant growth regulators under stress conditions, although they have unique role in plant metabolism and control processes of protein synthesis and cell division. There are some assumptions about the fast decrease after stress beginning and their later decrease. Thus in our research we have been investigated the dynamics of cytokinins (zeatin and zeatin riboside) in the leaves of two cultivars of winter wheat under high temperature stress.

It has been shown that under optimal temperature conditions (22-24°C) cytokinin content something varied, but under high temperature (38-40°C) these oscillations have been more sufficient: zeatin content rised in the first 30 minutes of exposition under high temperature and later something restored. Zeatin riboside dynamics had another character - it was increased after the start of stress and decreased in the second half of hour. Probably the pool of zeatin riboside is the reserve for zeatin in leaves but this reserve is not enough for the compensation of concentration of physiological active forma of cytokinins.

P0070. Structures parameters and contents abscisic acid in *Persicaria amphibia* (L.). Delarbree in different ecological conditions of growth

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In modern science there are few investigations in hormonal control of growth and development of plants which can grow for a long time in natural adverse conditions. That is why we have studied adaptive reactions of *Persicaria amphibia* (L) Delarbree by the example of comparative analysis structures parameter of aquatic and ground forms of a plant adapted to various moisture conditions and changes of the contents of an abscisic acid (ABA) at vegetative and reproductive organs. We took sprout height, quantity of internodes and leaves on a sprout, size of inflorescence and leaves, mass of wet and dry sprouts as data of our studying.

We discovered that in conditions of temperate moisture deficit there was higher percentage of ABA implants, smaller leaves and sprouts, lower data of wet and dry sprout mass. We consider all these factors to be adaptive reactions to the change of moisture conditions.

In our further study we plan to investigate peculiarities of *Persicaria amphibia* (L) Delarbree phytohormone complex during ontogenesis in different ecological conditions.

P0071. Role for aldehyde oxidases in the biosynthesis of indole-3-acetic acid and abscisic acid in *Zea mays*

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Aldehyde oxidase (EC 1.2.3.1) activities in the protein extracts from several tissues of maize (*Zea mays*) were detected by native PAGE and activity staining. The aldehyde oxidase signals were observed as triple bands, following general patterns that have been reported in many other plants, in maize kernels and young seedlings with slightly different tissue specificities. Among the many tissues, we concentrated on the aldehyde oxidase activities in the tip of primary roots where the aldehyde oxidase activities were clear. Efforts not only to measure the activities, but also to examine the gene expression were made. The major role for the enzyme has been known in abscisic acid biosynthesis in *Arabidopsis*. However, in maize the function of the enzymes is not yet definitely verified. Therefore, we discuss the possible correlations between the aldehyde oxidase activities and indole-3-acetic acid/abscisic acid biosynthesis in maize.

P0072. Phytohormones are involved in the pollen-pistil interactions during progamic phase of fertilisation in *petunia*

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The data obtained provide evidence for the involvement of ethylene (and ACC and two key enzymes in ethylene biosynthetic pathway, ACC synthase and ACC oxidase) together with other phytohormones (IAA, ABA, GA and cytokinins) in pollen-pistil interactions, controlling uninterrupted pollen tube growth after self-compatible pollination or its inhibition after self-incompatible

pollination. Endogenous levels of the above hormones in pistils, and their parts (stigmas, styles and ovaries) and growing *in vitro* pollen tubes were determined by HPLC. Ethylene production by pistil tissues was estimated by GC. The ethylene/ABA status of the stigma may be proposed to control the processes of adhesion, hydration, and germination of pollen grains, whereas the auxin/cytokinin status of the style may be involved in the control of pollen tube growth. Sharp differences in the ABA levels in stigma and cytokinin levels in style most likely reflect different abilities of pollen tubes to grow following compatible and incompatible pollination. These data suggest that ethylene, ABA and cytokinins appear to be implicated in RNase-based gametophytic self-incompatibility.

P0073. Characterizing cytokinin oxidase (CKX) throughout the development of R50, a pea mutant accumulating cytokinins

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Cytokinins (CK) are an adenine-based family of hormones, the content of which is regulated by cytokinin oxidase (CKX). The pea mutant R50 (*sym-16*) displays several traits indicative of increased CK content. This accumulation was recently confirmed by LC-MS-MS and may be a result of either reduced activity or expression of CKX. Biochemical activity was detected through development of R50 and wildtype (WT) by a colorimetric assay (Liberos-Minotta and Tipton, 1995). Activity in mature, dry and imbibed seeds of R50 was low but comparable to that of the WT. In contrast, a significant decrease in CKX activity was noted in the roots and shoots of R50 seedlings and mature plants, which mirrored the previously obtained CK profile. Using two recently published ESTs (Vaseva-Gemisheva *et al.*, 2003), we have begun to analyze the *Pisum sativum* CKX (PsCKX) gene family. Protein alignment and Southern blotting indicate that the PsCKX gene family is similar to those of *Arabidopsis* and maize in that it is a multi-gene family containing a conserved FAD-binding domain. The CKX expression profile throughout development is now being pursued to complement the biochemical data.

P0074. Cytokinins in tobacco chloroplasts

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Cytokinins (CKs) are plant hormones, which have a very close relationship to chloroplasts. We have reported the occurrence of a whole spectrum of cytokinins and cytokinin oxidase in tobacco and wheat chloroplasts (Benková *et al.*: Plant Physiol. 121, 245-251, 1999). Further we analysed CK levels by LC-MS in chloroplasts of transgenic plants with an altered CK metabolism (carrying an isopentenyltransferase *Pssu-ipt*, a zeatin-*O*-glucosyltransferase *ZOG1*, a cytokinin oxidase *AtCKX3* or a -glucosidase *Zm-p60.1* gene). The presence of the CK overproducing transgenes results in whole leaves in elevated levels of CK glucosides. But this increase was not found in isolated chloroplasts, which indicates that CK glucosides are accumulated outside chloroplasts, probably in vacuoles. The overexpression of CK catabolic *AtCKX3* gene decreased the CK content in chloroplasts. Chloroplasts isolated from plants overexpressing gene *Zm-p60.1* quickly cleaved added ZOG and thus affected the equilibrium between free and conjugated CKs.

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P0075. Growth Regulatory Activity of Parthenin as a Function of Time and Concentration

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Parthenin, a sesquiterpene lactone, is a natural constituent of *Parthenium hysterophorus*. Since sesquiterpene lactones, in general, possess growth regulatory activity, a study was planned to explore this property in parthenin. Effect of parthenin (25 to 500 µM) was assessed on the morphogenetic response of mung bean hypocotyls cuttings as a function of time period exposure (up to 96-h). Number of roots per hypocotyl cutting of mungbean increased significantly compared to control when treated with

lower concentrations of parthenin (25 and 50 µM) for lesser time. Even the length of roots was more compared to control. However, at higher concentration of parthenin (100 µM or more) number of roots per hypocotyl cutting either decreased or remained same depending upon the time of exposure and parthenin concentration. At 500 µM parthenin, a severe toxicity (the cuttings turned brown and were completely wilted) on the hypocotyls cuttings was observed even if exposed for a short time of 1h. Based on the study, it is concluded that parthenin exerts a growth regulatory response that is not only dependent upon concentration but also on time of exposure.

P0076. Quantification of endogenous ABA levels in normal and water-stressed *Nicotiana tabacum* L. leaves

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Immunoassay for abscisic acid (ABA) has been confirmed as the most sensitive and selective detection method for ABA. We have raised polyclonal antibodies (pAbs) specific against C1-immobilised (+)-*ct*-ABA conjugated to bovine serum albumin according to Weiler (1980). The pAbs isolated from rabbit serum by ammonium sulfate precipitation were characterised by ELISA and the affinity purified antibodies (ABA-1, ABA-2) were used for development of immunoaffinity chromatography. The polyclonal antibodies reacted preferentially with the (+)-*ct*-ABAmethylester and relatively well with (+/-)-*ct*-ABA glucosylester, (+/-)-*ct*-ABAmethyl ester, (+/-)-*ct*-ABA alcohol, (+/-)-*ct*-ABA aldehyd, (+/-)-*ct*-ABA, (+)-*ct*-ABA were also strongly reactive. Cross-reactivity with other compounds was negligible. The LC-ESI-MS and HPLC-ELISA have been used for comparative estimation of endogenous levels in immunoaffinity purified extracts of normal and water-stressed *Nicotiana tabacum* L. leaves.

P0077. Characterization of a plant chromatin-remodelling complex involved in auxin and cytokinin signalling

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Growth and development are extensively studied phenomena in multicellular organisms, and depend on the coordinated interplay between cell cycle progression, cell cycle exit and the onset of differentiation programs. In plants, the phytohormones auxin and cytokinin play central roles in the regulation of cell division and differentiation. Recent work in our laboratory demonstrated that the impact of both growth regulators on cellular identity, involves components of a chromatin-remodeling complex. Specifically, PROPORZ1 (PRZ1), a putative transcriptional adaptor protein, was shown to affect spatial and temporal expression of cell cycle regulators in response to variations of phytohormone concentrations. Progress in the characterization of the regulatory impact of PRZ1 on cell cycle regulator transcription and in the identification of additional components of the PROPORZ chromatin-remodelling complex will be presented. (work supported by FWF grant P15441)

P0078. Auxin plays a key role as intercellular signal in embryo development of European larch (*Larix decidua* Mill.)

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Somatic embryogenesis provides the opportunity to study embryo development from early to mature stages in the absence of maternal factors. Auxin transport inhibitors or -antagonists added to the culture medium caused alterations of the embryo phenotype correlating with investigations of excised zygotic and somatic embryos of angiosperm species. Similar morphological effects have been provoked by conjugating free IAA (35S::IAAL). The GH3 promoter has been shown to be auxin-inducible in *L. decidua* embryos and was used to visualize auxin distribution. An expression of the GH3::GUS gene was first detected in the late globular stage in the columnella cells and precursors. Exogenous auxin induced a heterogeneous expression pattern which changed during embryo development. GC-MS analysis of IAA levels showed a strong increase of IAA concentration during the transition stage from globular to bilateral symmetry which decreased later to the basal level of the earlier stages. The results indicate that specific patterns of auxin distribution combined with

an increase of IAA levels during the transition stage might be a prerequisite for establishing the embryo axis.

P0079. The effect of kinetin on the level of polyamines bound to thylakoidal membranes and activity of thylakoidal transglutaminase during the chloroplast differentiation in cucumber cotyledons

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In the early phases of chloroplast differentiation under light-condition, the content of putrescine (PU) and spermidine (SD) bound to plastid membranes was enhanced by kinetin treatment; conversely, the content of spermine (SM) was not affected. At 6h kinetin enhanced by 60% the SD level in comparison to the control. At 24h of light exposure, which represents the completion of the differentiated structure of cytokinin-induced chloroplasts in cucumber cotyledons, the PAs binding to plastid membranes decreased.

It was verified that the binding was at least in part due to a transglutaminase (TGase) conjugation. Spermidine was conjugated to thylakoidal membranes with higher efficiency than PU. The kinetin pre-treatment enhanced the SD conjugation in the early phases of thylakoid differentiation. The net decrease of TGase activity at 24h of experiment was correlated with the decrease in the content of PAs bound to the thylakoids.

The novelty of the present research is the finding that PAs, at least in part are covalently linked by a TGase activity stimulated by cytokinins during the early phases of chloroplast photodevelopment.

P0080. Effect of culture media and phytohormones on organogenesis and proliferation of the shoots in *Codiaeum aucubifolium*

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The ongoing way of multiplication in *Codiaeum aucubifolium* is cutting of the leafy stems, which is not completely successful and it could increase the number of infected plants. Therefore, we used plant tissue culture for proliferation. First, the explants of leaves and internodes were cultured on 2, 4-D (1mg/lit) supplemented media including: MS, An, and MC. The An medium was the best in regard to the production of big and fresh calli. Then, callogenesis was studied only in An by adding different concentrations of BAP (0, 0.5, 1, 2, and 4 mg/lit) with various dilutions of one of the auxins including: IAA, 2, 4-D or NAA. Callus induction was greatest in IAA (4mg/lit) + BAP (1mg/lit), NAA (2mg/lit) + BAP (1mg/lit) and 2, 4-D (1mg/lit) alone. To evaluate the organogenesis, the greenest cultured Calli were transported to the media supplemented by various concentrations of different cytokinins (2ip, BAP, Kin, and coconut milk) and above auxins. The most excellent organogenesis was seen in coconut milk (20%) + 2, 4-D (1mg/lit). The best combinations for elongation and proliferation of shoots were 2ip (1mg/lit) + IAA (0.5mg/lit) and IAA (2mg/lit) + BAP (1mg/lit) respectively.

P0081. Influence of Photoperiod and Gibberellic acid(GA₃) on the growth and flowering of cowpea [*Vigna unguiculata*(L)WALP]

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The effects of different levels of photoperiod (P;h) and 50ppm gibberellic acid (GA₃) were studied on the vegetative growth, phenology and yield of a selected, unimproved but popular cowpea variety - Kanannado. The different photoperiods used were 10, <12.5, >12.5 and 14h. Greater growth was observed in the long day lengths (P > 12.5 and P = 14h) except for hypocotyl length and stem circumference. Flowering and pod maturity were enhanced under short photoperiods (P= 10 and P< 12.5h) while Yield was higher in the long photoperiods (P>12.5 and P=14h). The GA₃ treatment stimulated an increase in the growth of the cowpea, enhanced its flowering and pod maturity by 1- 5days and increased yield under all the photoperiods. This

study indicates that as efforts are being made towards maximizing yield of

this crop in the dry season (with irrigation), efforts should be made on increasing its vegetative growth. Foliar application of GA₃ appears promising and more work should be carried out to determine the optimum concentration of the hormone as well as explore the possibility of using other hormones and/or combined hormones.

P0082. Diversity in salicylic acid effects on growth criteria and different indole acetic acid forms among faba bean and maize

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All SA doses (1-5 mM) tended to induce increases on faba bean growth criteria. After 30 days from treatment, there was a progressive increase in the fresh and dry weights of stem, leaves and whole plant as well as number and fresh weight of kernels by increasing SA level. Application 3-5mM resulted in a remarkable reduction in maize growth. Free, ester, amide, bound and total IAA were determined by using HPLC. Free IAA in leaves of faba bean and maize plants reached about 31 and 14% of the total IAA, respectively. Spraying faba bean plants with SA tended to cause increases in free IAA and decreases in ester IAA, after 3 days from treatment. Values of free and ester IAA reached 113% and 44% of control by using SA at 4mM, respectively. As for maize, all SA doses caused decreases in free IAA and increases in ester IAA. SA did not induce a great consistent effect on amide-IAA and total IAA, while, changes in ester form were in opposite manner to changes in free IAA, in most cases, meaning that SA interfere with IAA-conjugation.

P0083. 6-Benzylaminopurines - an interesting cytokinin alternative for plant biotechnology and agriculture

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Our search for naturally occurring aromatic cytokinins in plants using LC-MS methods led recently to the discovery of several new plant hormone substances - methoxybenzylaminopurines (Tarkowská et al., 2003). Subsequently, a group of their synthetic analogues has been prepared and characterized. Various aspects of their biological activity (cytokinin effect studied on molecular (receptor) level and *in-vitro* as well as in field experiments, anti-cancer properties etc.) have been investigated. To study these interesting compounds in more details, rapid and highly efficient method for their isolation from complex biological matrices, based on immunoaffinity chromatography, has also been developed.

Studied compounds are proposed to be new endogenously occurring members of the aromatic cytokinin subgroup, which might have specific physiological functions. Mechanism(s) of their anti-tumor activity will be further studied using newly developed analytical methods.

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P0084. The role of ABA and NO in the drought stress acclimatisation mechanisms of wheat genotypes

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The biosynthesis of the plant stress hormone abscisic acid (ABA) is regulated not only by osmotic stress but also by the level of ABA itself. Two water stress tolerant wheat varieties (*Triticum aestivum* cv. Kobomugi and GK Óthalom) were exposed to 400 mOsm polyethylene glycol (PEG) and the changes in water potential, stomatal conductance, ABA level and aldehyde oxidase activity (AO, a key enzyme of ABA biosynthesis) was estimated. Water potential values decreased substantially in the leaves of GK Óthalom, and remained constant in cv. Kobomugi. We found a higher increase in ABA concentrations and AO activities in cv. Kobomugi than in cv. GK Óthalom plants. ABA induces stomatal closure by activating several different signal transduction pathways. Stomatal conductivity measurements suggested that guard cells responded sooner to osmotic stress in Kobomugi than in Óthalom. The level and the distribution of NO during the osmotic

stress, determined by DAF-2 DA fluorescent probe staining showed different patterns in the two genotypes. Our results show that the two wheat lines possess different drought stress acclimatisation strategies.

P0085. Effect of ethylene on the seed germination of halophytes

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Seeds of halophytes usually fail to germinate under unfavorable conditions, such as too high salinity but remain viable and germinate upon the removal of environmental constraints. In contrast many seeds are unable to germinate, or do so with difficulty, when placed under favorable conditions, and they are considered as dormant seeds. This dormancy could be due to inactive embryo, impermeable seed coat or imbalance in hormone concentration etc. Ethephon -an ethylene releasing compound-stimulates germination of dormant and non-dormant seeds, although in some case they inhibit or do not affect germination. Ethylene removes primary dormancy, secondary dormancy and light induced dormancy. Salinity, high temperature, and stress caused by osmoticum could be reduced by ethylene. Ethylene shows a differential affect in reducing the stress caused by salinity during germination of halophytes. Germination inhibition caused by salinity could be partially or completely alleviated by ethylene while in other ethylene may not have any effect or a negative effect on seed germination. Variation in halophyte seed germination response to ethylene will be discussed.

P0086. The flowering of *Dendrobium* isolated shoots: the effects of thidiazuron on the endogenous levels of cytokinins and IAA

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Flowering is a unique event in the developmental cycle of a plant. Different internal as well as environmental factors are known to participate in the various steps that lead to flower formation. This study reports the effects of TDZ on the endogenous levels of Z, [9R]Z, iP and [9R]iP as well as some structural changes in the apical meristem of *D. Second Love* shoots during flower induction and initial development *in vitro*. The obtained results revealed that the addition of 1.8 µM TDZ to the culture medium had a profound effect on the endogenous cytokinins and IAA levels of the explants, when compared to those grown on TDZ-free medium. A significant increase in total cytokinins (especially [9R]iP and [9R]Z) and IAA on the 5th day of culture on TDZ-enriched medium was associated with flower induction, and a second increase in the level of these hormones after 25 days of culture was related to flower development. The histological changes detected in the shoot apical meristem of explants grown in the presence of 1.8 µM TDZ during 30 days of culture are also described. Based on our findings, both auxin and cytokinins were involved with the floral transition of *D. Second Love in vitro*.

P0087. Quantification of free and total salicylic acid in plants by solid-phase extraction and isocratic high-performance anion-exchange chromatography

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Salicylic acid (SA) is an important plant hormone with a key role during plant resistance responses. The resistance mechanisms involving SA signalling include hypersensitive response, systemic acquired resistance, pathogen-related (PR) gene expression, oxidative burst, and programmed cell death. Furthermore, SA seems to induce resistance to viruses by inhibition of viral replication and systemic movement.

Despite its central role in plant defence the levels of SA remain often uncharacterised due to requirement of special equipment and the time consuming nature of current protocols for SA quantification. Here we describe a simple, novel method for reliable quantification of SA with a standard HPLC equipment that can be easily applied to different plant species by many

laboratories. The techniques presented here combine several advantages including highly selective sample preparation, a novel, improved internal standard, a reduced analysis time and increased sensitivity. Thus, sample size can be scaled down compared to current HPLC methods - an invaluable benefit for applications with limited plant material.

P0088. Structures parameters and contents abscisic acid in *Persicaria amphibia* (L). Delarbre in different ecological conditions of growth

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In modern science there are few investigations in hormonal control of growth and development of plants which can grow for a long time in natural adverse conditions. That is why we have studied adaptive reactions of *Persicaria amphibia* (L) Delarbre by the example of comparative analysis structures parameter of aquatic and ground forms of a plant adapted to various moisture conditions and changes of the contents of an abscisic acid (ABA) at vegetative and reproductive organs. We took sprout height, quantity of internodes and leaves on a sprout, size of inflorescence and leaves, mass of wet and dry sprouts as data of our studying.

We discovered that in conditions of temperate moisture deficit there was higher percentage of ABA implants, smaller leaves and sprouts, lower data of wet and dry sprout mass. We consider all these factors to be adaptive reactions to the change of moisture conditions.

In our further study we plan to investigate peculiarities of *Persicaria amphibia* (L) Delarbre phytohormone complex during ontogenesis in different ecological conditions.

P0089. Glycoproteins from sugarcane cv. Ja 60-5 regulate cell polarity of *Ustilago scitaminea* teliospores as a defence mechanism.

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Saccharum officinarum, cv. Ja 60-5 is a variety of sugarcane with a moderate susceptibility to smut disease caused by *Ustilago scitaminea*. Sugarcane produces glycoproteins that accumulate in the parenchymatous cells of stalks and that have been described as factors of resistance to smut. Glycoproteins obtained from a resistant cultivar of sugarcane show inhibitory effect on processes related to viability and development of the pathogen such as teliospore polarization and germination and growth of the micellium. In this work we have studied the effect of glycoproteins obtained from a susceptible cultivar, on smut fungus. Smut teliospore aggregation is observed when glycoproteins of both healthy and inoculated plants are used. However, although the amount of glycoprotein is higher in inoculated plants, these glycoproteins show an ability to bind to the teliospore cell wall receptor and to inhibit cell polarization and germination lower than those obtained from healthy plants. So, smut development seems to be able to change the pattern of glycoprotein action, leading to a higher level of susceptibility in this variety of sugarcane.

P0090. Physiological response to inoculation of *Cryptococcus laurentii* in harvested peach fruit

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We examined the effect *Cryptococcus laurentii* and/or challenge by *Penicillium expansum* induced of harvested peach fruit on changes in the ethylene productions, the activities of superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), phenylalanine ammonialyase (PAL), the polyphenol oxidase (PPO), and the contents of malondialdehyde (MDA). The results showed that *C. laurentii*-treated significantly reduced the decay and inhibited the ethylene production, and the activities of SOD, CAT and PAL, enhanced the activities of POD and PPO, and MDA contents compared with wound control fruits. Compared with infected by *P. expansum* only, pre-inoculated with *C. laurentii* and challenged by *P. expansum* inhibited the activities of PPO and POD and slightly inhibited the ethylene production (day 3), maintained relatively higher lever of SOD activity, and induced a rapid and great increase in PAL activity in wound inoculated fruits. At the increasing distances (at +45-and +90mm), the higher

activities of SOD, PAL, and PPO, and the MDA contents were observed compared to that at +2mm from *C. laurentii*-inoculated site.

P0091. Avaluation of pathogenesis isolated from *Lasiodiplodia theobromae* in carica fruit (*carica papaya*) and activity agaisnt actinomycetes in the control of lasiodiplodiose

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Pathogenesis of *L. theobromae* was evaluated to the papaya, and the activity, *in vitro* and *in vivo*, of actinomycetes against *L. theobromae*. The pathogenesis of *L. theobromae* was tested with seven isolated of different hosts. Of the seven isolated tested, two (URM-3184 and URM-2704) were the more pathogenics. Of the 40 tested actinomycetes, 17 went assets. The best times of fermentation was 72, in medium ISP-2. The result of the test of unit of Waksman showed that the active substance is contained predominantly in the cell mass. The ethanol in pH 7, as the best solvent extractor of the same. Three experiments *in vivo* were accomplished; for each experiment three treatments were accomplished (preventive, curative and simultaneous), with three repetitions each. Isolated URM-2704 was more sensitive than URM-3184, to the antagonistic effect of the actinomiceto DAUFPE-11470. The experiment 2, presented total protection until, for isolated URM-2704 in the preventive treatment.

P0092. Sources of the free radical nitric oxide during Arabidopsis thaliana-Pseudomonas syringae interaction

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Despite the many processes controlled or induced by nitric oxide (NO) in plants, the molecular mechanisms responsible for the synthesis of this radical remain controversial and a NO synthase (NOS)-like enzyme and nitrate reductase (NR) are claimed as potential sources. Here, we used wild-type and double mutant plants deficient in the NR enzyme to investigate NO production in *A. thaliana* in response to *P. syringae* pv. *maculicola* (Psm). Production of citrulline from arginine following the inoculation of Psm was increased in *A. thaliana* leaves from wild-type and NR-deficient plants. EPR experiments showed a much higher NO production, in both genotypes, that was dependent on nitrite rather than on arginine or nitrate. Electron transport by the mitochondrial respiratory chain was essential for this NR-independent NO production. *In situ* NO production was substantially increased in wild-type plants but not in mutant, following a challenge with Psm, as measured with the fluorescent indicator DAF-2DA. Concluding, NOS, NR and a mitochondrial-dependent nitrite-reducing activity are involved in producing NO for *A. thaliana* defense against *P. syringae*.

P0093. First Report of Pathogenicity of Binucleate Rhizoctonia spp. Causing Corm and Stem Rot of Gladiolus

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Fungi with Rhizoctonia-like mycelia were isolated from root and stem of Gladiolus (*Gladiolus hybrida* L.) grown in commercial glasshouse in Mahallat, Iran, during the summer and fall of 2003. On the basis of hyphal characteristics and nuclear number, twenty-three isolates of *Rhizoctonia* spp. were obtained from infected corms and stems. Of the 23 isolate, 9 had binucleate and 14 had multinucleate vegetative hyphal cells. Representative isolates of binucleate *Rhizoctonia* spp. were characterized for anastomosis, optimum temperature *in vitro*, and virulence on gladiolus. Isolates of binucleate *Rhizoctonia* failed to anastomose with tester isolates of anastomosis groups (AG)-A through -S (not including AG-J and AG-M). Growth rate of binucleate *Rhizoctonia* spp. was more rapid than *R. solani*. Five isolates from each group caused severe corm rot and mortality of plant during rooting. This is the first detailed report of corm and stem rot disease of gladiolus caused by binucleate *Rhizoctonia*. Further field studies are needed on the ecology of *Rhizoctonia* spp. to formulate steps for controlling the disease.

P0094. Analysis of salicylic acid - inducible protein-DNA interaction using chromatin immunoprecipitation in tobacco leaves

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The as-1 element, originally found in the Cauliflower Mosaic virus, is inducible by salicylic acid, auxin and also by pathogen attack. Several as-1 like elements were found in plant promoters of pathogen defense genes like the "early" response gene Nt103 (a glutathione-S-transferase) or the "late" response gene PR1a encoding pathogenesis related protein 1a.

Binding studies with tobacco nuclear extracts using EMSA revealed recognition of the as-1 element by TGA factors TGA2.1 and TGA2.2, members of the bZIP transcription factor family.

To investigate the *in vivo* interaction between TGA factors and as-1 like promoter elements we have developed a chromatin immunoprecipitation assay (ChIP) for tobacco leaves. Establishment of the technique was validated using the tetracyclin (Tc) - inducible expression system in tobacco plants allowing successful analysis of two different conditions (+ / - Tc).

ChIP experiments with transgenic tobacco plants carrying the reporter construct as-1:GUS showed a constitutive binding of TGA2.2 to the native as-1 element and inducible binding to the as-1 like element of the PR1a promoter by SA treatment.

P0095. Assessment of root-organ cultures to study the mycorrhizal phenotype of pea nodulation mutants.

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Our lab is interested in studying the mycorrhizal (myc) phenotype of a number of pea nodulation mutants using root-organ cultures. To assess this technique, we used the mutant E107 known to accumulate Fe in its shoots, exhibit a low nodulation phenotype, and display a low myc phenotype when associated with *Glomus aggregatum* in the soil. Roots were used either as a root-organ culture or as a chimera (roots transformed / shoots non transformed). Whereas the primary root was cut before *Agrobacterium rhizogenes* was applied for the former, it was pricked with a needle for the latter. Once transformed, roots were transferred to Phytigel[®] medium, and hyphal plugs with *Glomus intraradices* spores were placed next to them. Spore germination and infection were followed with light microscopy. Interestingly, E107 forms numerous mycorrhizae in root-organ cultures. However, the limits of the cultures will be ascertained only when the chimera's roots are examined. Indeed, the myc⁺ phenotype of E107 root-organ cultures could be explained either by the excision of the shoot (removing either the accumulated Fe or a potential shoot inhibitor), or by a more competitive fungus.

P0096. Molecular and biochemical studies to elucidate fireblight (*Erwinia amylovora*) resistance in apple (*Malus* spp.)

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Erwinia amylovora, the causal agent of the necrotic disease fireblight infects most members of the Pomoideae such as pear and apple. Differentially expressed genes (isolated via cDNA-SSH) of fireblight resistant and susceptible apple are screened to characterize genes involved in pathogenesis and resistance to fireblight. In addition isoflavonoids are important phytoalexins and have been shown to play a role in disease resistance. The gene expression patterns of phenylalanin ammonialyase (PAL), chalcon synthetase (CHS), dihydroflavonol reductase (DFR), flavanone 3-hydroxylase (FHT) of moderate resistant cv. Rebella and Reka were compared with the susceptible cv. Idared and with the resistant species *Malus robusta*. The temporal and spatial expression patterns of PAL, CHS, FHT, and DFR were investigated via *in situ* hybridisation, *in situ* PCR and real time PCR. The results show increased transcription of CHS and PAL in *M. robusta* corresponding also with high enzyme activities.

P0097. The composition and function of xylem sap proteins (XSP) during weeping period of the grape *Vitis vinifera* L.

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At the beginning of plants vegetation, xylem sap contains organic compounds that might be involved in root-to-shoot communication. To clarify the physiological functions of proteins in xylem sap, we studied the composition and biochemical activity of XSP of *V. vinifera* during its weeping period. SDS-PAGE showed at least 40 visible protein bands in a molecular mass range from 10 to 100 kD, whereas 2DE revealed more than 200 XSP. The spectrums of XSP from different *V. vinifera* varieties during tree years of analysis were qualitatively constant and quantitatively different. This suggests that XSP are not the products of degradation. Most of XSP are likely to be involved in redox regulation and to defense against biotic and abiotic stimuli. In support of this assumption are results of enzyme assays, which showed that XSP of *V. vinifera* have well delineated protease, - amylase, and peroxidase activities. We also found that the part of XSP are lectins, which specifically bind D-glucose and D-galactose. It is known that lectins are implicated in cell recognition and their being in xylem sap is the strong argument supporting the signaling functions of XSP.

P0098. Gene expression of IAA-amidohydrolases from *Brassica rapa* during clubroot disease

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Clubroot disease is caused by the obligate biotroph organism *Plasmodiophora brassicae*, who infects roots of all members of the Brassicaceae. The infected root show abnormal cell enlargement and cell proliferation, correlated with an increase in IAA. One source of free IAA is the release from conjugates with amino acids, catalyzed by IAA-amidohydrolases. We isolated cDNA fragments with homology to IAR3, ILL2, ILL6, ILL3 and ILR1 out of different tissues from *Brassica rapa* and full length cDNAs from the following amidohydrolases like genes: IAR3, were we found 2 different clones varying in the 5'-region of the genes, ILL2 and ILL6 which is placed in a separate group in the dendrogram of the IAA-amidohydrolases. Expression studies of the corresponding genes in infected and uninfected roots using Real Time RT-PCR revealed a differentially and very stage specific regulation. Heterologous expression studies in *E. coli* revealed first results for the in-vitro enzyme activity.

P0099. Pathogenesis-Related protein -1 Transcript Expression in Response to Salicylic acid in Potato

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Pathogenesis-Related proteins (PR) are defense proteins that express during pathogen attack. The effect of Salicylic Acid (SA), an important signal molecule in plant defense, was investigated on PR-1 transcript expression in potato. Potato leaves were treated with 1mM, 2.5mM and 5mM of SA. There was a little PR-1 transcript expression in the controls from 3 to 48 hours and PR-1 transcripts accumulated after 6 hours of treatments only at 2.5mM of SA and after 12 hours at 1mM of SA. Similar levels of PR transcripts accumulation were observed at 1 mM and 5 mM of Salicylic Acid from 24 to 48 hours. Electrophoresis of extracted DNA from the controls and treated ones showed no fragmentation after 24 hours, but some similar smears were appeared in the treated leaves in comparison to the controls. It seems that basal expression of PR-1 may contribute to make potato leaves resistance to DNA fragmentation after 48 hours in the controls. The data indicate that 2.5 mM of Salicylic Acid is critical concentration in inducing PR-1 expression in potato leaves. Therefore, PR-1 might be a signaling protein that makes cells more resistance to some stresses in Salicylic Acid - dependent pathway.

P0100. Tobacco bZIP transcription factor TGA2.2 and related factor TGA2.1 have distinct roles in plant defense responses and plant development

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Salicylic acid (SA) plays a crucial role as signaling molecule in plant defense reactions. TGA transcription factors bind to *activating sequence-1 (as-1)*-like elements, SA-responsive *cis* elements found in promoters of "immediate early" and "late" SA-inducible genes. Our earlier work has shown that the main component of tobacco *as-1*-binding complex ASF-1 is TGA2.2. TGA2.1, which differs from TGA2.2 by being able to activate transcription in yeast, constitutes a smaller fraction of the complex. Both TGA factors interact with NPR1, a protein essential for SA-inducibility of "late" genes. By using dsRNAi mediated gene silencing we demonstrate that reduced amounts of TGA2.2 lead to less ASF-1 activity and to decreased inducibility of "immediate early" and "late" genes. In contrast, reducing the amount of TGA2.1 had no effect on the expression of the analysed genes. TGA2.1 dsRNAi plants developed sepal like stamens indicating a regulatory role in defining organ identity in tobacco flowers.

P0101. Endopolygalacturonase from a saprophytic fungus releases elicitors of phytoalexin response from plant pectin

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We investigated the capacity of a saprophytic fungus, *Mucor ramosissimus*, to synthesise endopolygalacturonases (EndoPGs) capable of degrading cell wall pectins from *Palicourea marCGravii*, a tropical forest native Rubiaceae where the spores of the fungus have been found. The capacity of products of hydrolysis of pectin by this enzyme to elicit defence response in soybean and in other Rubiaceae species was also analysed. The fungus is capable of releasing pectinases to the culture medium when pectin is supplied as carbon source. The enzyme, purified by ion exchange chromatography and preparative isoelectric focusing presents pI 9.8 and optimal activity at pH 5.0 at 30 °C. The products of hydrolysis of different pectic substrates by the action of this endoPG elicit in different extents the phytoalexin production in soybean cotyledons and in the leaves of *P. marCGravii*. These results suggest that, besides the role in recycling organic matter, saprophytic fungi can also play an important role in the induction of defensive mechanisms in plants growing under natural environmental conditions by enhancing their unspecific resistance against pathogens.

P0102. Use of 3TAC (Trichoderma) in the clean tobacco growing.

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3TAC, is a biotechnological product, based on live trichodermas in all of its development stages, with its water soluble metabolites, powder, 100% organic made in Chile by Avance.

Tobacco growing problems: it is developed in a water environment, what favours the growth of fusarium, phytium, and a fungus complex that causes damping off. To do this cultivation it is necessary to apply chemical fungicides. The restrictions to the traceability and the need of having cleaner cultivations, made easier to use the 3 TAC on 3000 trays during season 2003-2004. Result: 3 applications of 3 TAC to the cultivation (peat, pre forst cut and second cut) replace 9 applications of chemical fungicides. There was a total control of pathogens, a greater % of germination and of spelling strenght. The release of auxine pulses from 3 TAC to the plants allowed a fast cicatrization after the pruning and a null development of pathogens, in comparison to little plants treated with traditional chemicals .

P0103. Influence of catenarin on cell cycle of wheat and triticale root tissue

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Catenarin (1,3,5,8-tetrahydroxy-6-methyl-anthraquinone) was found as major compound among anthraquinone derivatives produced by *Pyrenophora tritici-repentis* (Died.) Drechsler. Ability to biosynthesis of this metabolite is strain depended, and varied

from traces up to 400 ppm. *P.tritici-repentis* is world - wide distributed polyphagous fungus recognized on numerous Poaceae and responsible for tan spot of wheat and triticale. It is mostly a leaf pathogen but it is also seed transmitted and can infect cereal roots. In performed studies influence of catenarin on cell cycle of wheat and triticale root tissues was tested. After 48 hours of treatment the metabolite at 5 and 50 ppm arrested cell division. In case of the both mentioned cereals, cell accumulation at the G2 phase was observed. Root tip squashes showed fewer mitotic index in treated than in catenarin untreated plants. No effect was observed after 24 h exposure time.

P0104. Effect of non-nodulating soybean seed lectin on nodulation of soybean plants by *Bradyrhizobium japonicum*

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As a result of germination the non-nodulating soybean seeds released haemagglutinating protein (lectin) in environment. The non-nodulating soybean seeds lectin was extracted and purified. Pretreatment of *Bradyrhizobium japonicum* 634b with either 10^6 or 10^7 the lectin molecules per bacterium resulted in enhancement of nodule formation at 11 days after inoculation. This effect is also seen after preincubation of the nodule bacteria with nodulating soybean seed lectin. But pretreatment with nodulating soybean seed lectin increased nodule number during 30 days after inoculation. The percentage of nodulated plants within first days of plant growth increased to 82% when they were inoculated with treated nodule bacteria. Interestingly, non-nodulating soybean seed haemagglutinin promoted mainly formation of nodules on primary roots at 16 days after inoculation. These findings suggest that the non-nodulating soybean seed lectin can be kind of the substance, which is able to modulate legume-Rhizobium symbiosis formation.

P0105. Comparison of the migration of gfp labeled *Erwinia amylovora* on blossoms of apple cultivars of different susceptibility to fire blight

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Detached flowers of susceptible and tolerant apple cultivars were inoculated with gfp labeled *Erwinia amylovora*. Migration of the bacteria and progression of the infection has been detected. The traits of the hypanthium surface are of prominent importance in the progression of the infection. The wrinkled surface, the convex shape of the outer epidermal cell walls with thin cuticle and the sunken stomata helped to preserve a water film for a longer period providing medium for the motility of the bacteria in the susceptible cultivar. Large bacterium aggregations emerge gradually in the intercellular spaces of the glandular tissue, in the adjacent parenchyma of the hypanthium wall, in the wall of the ovary and in the pedicel. In the blossoms of the tolerant cultivar bacteria were restricted to small water droplets on the flat and waxy surface of the hypanthium and only a few were able to enter the tissues. Low level of bacterium aggregation and migration was found in the intercellulars of the subepidermal tissues of the hypanthium. In the tissues of the ovary wall and pedicel bacteria were not detected.

P0106. Effects of OTC-treatment on subcellular glutathione contents and symptom development during ZYMV-infection

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Styrian oil pumpkin seedlings (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* GREB.) were treated with 1mM OTC (L-2-oxothiazolidine-4-carboxylic acid) to artificially increase cellular glutathione contents. Biochemical and immunocytological investigations revealed that two days after OTC-treatment glutathione contents were increased in all investigated organs (roots, cotyledons and the first leaves) of up to 2.5-fold in peroxisomes of cotyledons. Seedlings were infected with Zucchini Yellow Mosaic Virus (ZYMV) 10 days after OTC-treatment and symptom severity was evaluated three weeks later. 70% of ZYMV-infected plants treated with 1mM OTC prior to infection showed no signs of symptoms although virus particles were detected in leaves and roots with negative staining. Only 30% of the plants showed signs of ZYMV-infection like yellowing, minor stunting and first signs of mosaic patterns. No effects of OTC-pretreatment on glutathione contents were observed at that time within these plants.

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P0107. Leaf beta-1,3 glucanase and chitinase activities as defense mechanisms of sugarcane plants against pathogens

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Leaf scald produced by *Xanthomonas albilineans*, and smut, caused by *Ustilago scitaminea* are the two major diseases of sugarcane plants. Plants develop several enzyme activities as a defense mechanism against pathogen. Some hydrolases, proteases and enzyme related to phenolics acid metabolism are included in these defense mechanism.

We are used five different cultivars of *Saccharum officinarum* with different sensitivity against both pathogens. Using leaves of these plants, -1,3-glucanase, and chitinase were assayed in healthy and experimentally infected plants. Enzyme activities were measured using intact discs of leaves permeabilized with 4% iso-propanol. Laminarin was used as a substrate for glucanase whereas commercial chitin was the substrate of chitinase. L 55-5 produces the highest -1,3-glucanase activity after infection with *X. albilineans*, whereas My 55-14 contains the lowest level of this enzyme. Ba 42-231 shows the highest levels of chitinase activity after smut infection whereas My 55-14 newly develops the lowest enzyme activity.

P0108. Biological properties and nucleotide sequence of a begomovirus from *Malvastrum coromandelianum* (L.) Garcke

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A yellow-veined *Malvastrum coromandelianum* (Malvaceae) was collected in Thailand. Transmission tests showed that the symptom could be transmitted to healthy plants by grafting and by whitefly (*Bemisia tabaci*) in persistent manner. Southern blot hybridization using begomovirus DNAs as probes gave positive results in DNA extracted from infected plants, indicating that this putative virus was a bipartite begomovirus. Insect transmission tests showed that individual whitefly could transmit the virus to 30% of tested plants. Once the insects acquired the virus, they could transmit as long as 12 days or more. Host range study on 21 plant species showed that the virus could not multiply in 18 species tested. In *Nicotiana benthamiana* and tomato infected with the virus, leaf curl symptom was detected. Although infected tobacco did not show visible symptom, Southern blot hybridization indicated the presence of the virus. Nucleotide sequence comparison of the common region showed that the virus was slightly similar to *Cotton leaf curl Rajasthan virus* (79.63% identity). Phylogenetic analyses revealed that the virus was closely related to the Old World viruses.

P0109. Effect of sugarcane glycoproteins on the production and activity of UDPG-dehydrogenase from *Xanthomonas albilineans*.

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X. albilineans produces a xanthan like polysaccharide consisted of a basal tetramer composed by two molecules of fructose, one mannose rest and a final glucuronic acid. *Xanthomonas* produce an active UDPG dehydrogenase which is seen as a virulence factor. Sugarcane plants are able to produce glycoproteins as a response to parasitic invasions. They are grouped in two classes as a function of their molecular mass. HMMG (high molecular mass) and MMMG (mid molecular mass). The addition of HMMG or MMMG from 22 month-old stalks of Ba 42231 cv, highly sensitive to leaf scald, did not inhibit the initial loss of enzyme activity but strongly enhanced this activity from 15h (for HMMG) or 21h (for MMMG) to 24h culture. The enzyme was completely inhibited when HMMG and MMMG isolated from 22 months-old stalks of My 5514, highly resistant to leaf scald, were added to the culture media. This enhancement of UDPG-DH activity effected by HMMG and MMMG from Ba could be due to an activation of a pre-existent enzyme by sugarcane glycoproteins or to an activation of the enzyme synthesis. To study this effect we included in the culture media the inhibitors 8-azaguanine, chloramphenicol and piperine.

P0110. Lysozyme-like bacteriocin production from *Gluconacetobacter diazotrophicus* against *Xanthomonas albilineans* (scald leaf pathogen) in stalks of sugarcane

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Leaf scald is a major disease of sugarcane which occurs in most sugarcane-producing countries and continues spreading. *Xanthomonas albilineans* is the pathogen. Gum exudates, which occluded bundle vessels, were observed in the ultrastructure of sugarcane scalded leaves. The gum was identified as a xanthan-like polysaccharide.

Gluconacetobacter diazotrophicus is a sugarcane endophyte which produces a lysozyme-like bacteriocin in liquid medium that inhibits growth of *X. albilineans*.

7 months-stalks of *Saccharum officinarum* were inoculated with the endophyte, or with the pathogen or with both bacteria and maintained for 11 days at 30° C. Extraction of xanthan was carried out from juice of these stalks, filtered through Sephadex G-10 and G-50 columns and carbohydrates were monitored. Main fractions of carbohydrates were hydrolyzed with 6 N HCl at 80° C overnight, dried and ground with cold 80% (v/v) ethanol. After evaporation to dryness, xanthan precipitates were analyzed by capillary electrophoresis.

The aim of this work is production of bacteriocin from *G. diazotrophicus* in sugarcane to establish a possible mechanism of biological control of scald leaf.

P0111. Localisation of *Xanthomonas albilineans* within inoculated leaves of sugarcane

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Leaf scald, caused by *Xanthomonas albilineans*, is a major disease affecting sugarcane and has been found in 57 countries. The initial characteristic symptom of disease is a white-yellowish streak on the leaf along the main veins. The streaks may later become more enlarged and affected leaf becomes wilted and necrotic. The white-yellowish streaks developed large bulliform cells and a gum exudates which occluded phloem and bundle vessels. The gum was purified from juice obtained from scalded stalks and was identified as a xanthan-like polysaccharide.

To localise *X. albilineans* in sugarcane leaves, 10-months leaves of *Saccharum officinarum* were inoculated with 0,6 mg dry weight of *X. albilineans* with a needle in the main vein and maintained in green house conditions. Small pieces (1 mm x 1 mm) of sugarcane leaves were taken at 1, 2, 5 and 7 days and to 2 cm above and below from the point of inoculation and infiltrated in LR White resin. Semithin sections were used for immunogold labelling (IGL) or stained with metilen blue for conventional light microscope. Ultrathin sections for transmission electron microscope were used for IGL.

P0112. Activation of actinorhizal nodulin gene promoters in legume nodules of *Medicago truncatula*

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Two groups of plants, legumes and actinorhizal plants, are able to enter root-nodule symbioses with nitrogen-fixing bacteria, rhizobia and *Frankia*, respectively. In spite of the differences between actinorhizal and legume symbioses, the two systems share common aspects, especially regarding the infection process and nodule functioning. Our main goal is to compare the two symbioses at the molecular level. This way, we expect to identify some of the common aspects determining the capacity to develop nitrogen-fixing root nodule symbioses and/or to provide insight about the different strategies used by plants to achieve symbiotic nitrogen fixation. Here we report on the study of two actinorhizal nodulin gene promoters, one from *Casuarina glauca* and one from *Datisca glomerata*, in the legume *Medicago truncatula*. The results indicate that at least part of the transcriptional environment in plant cells infected by endosymbiotic bacteria is conserved between legumes and actinorhizal plants.

P0113. Influence of lectin on the efficiency of symbiotic system soybean - *Bradyrhizobium japonicum* under different mineral nitrogen supply

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The influence of preliminary incubation of nodule bacteria *Bradyrhizobium japonicum* (active strain 634b and non-active strain 604k) with homologous soybean lectin on the infectivity, nitrogen fixing activity of root nodules and development of soybean plant (*Glycine max* (L) Merr.) under different mineral nitrogen supply were studied. It is shown that preliminary incubation of nodule bacteria with lectin increases the nitrogen fixing activity of soybean nodules that were formed by the active strain 634b. And on the contrary, there has been revealed the tendency of inhibiting the activity of nodules that were formed by non-active strain 604k under the conditions of different supply with mineral nitrogen. As a result of our research it has been established that the influence of preincubation of nodule bacteria with lectin on the efficiency of symbiotic system depended on the strain activity and was limited by volume of mineral nitrogen in substrate.

P0114. Rhizobial isolates effectiveness on cowpea

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In order to identify rhizobial strains with higher biological N₂ fixation capability in cowpea 15 strains isolated from non-inoculated cowpea plants grown in the savannah soils of Monagas State, Venezuela, were evaluated. A completely randomized design with four replicates was used. Two control treatments were included, one non-inoculated and N-fertilized and the other non-inoculated and non-fertilized. The plants were cultivated in plastic pots with 4 kg of sterilized fine granite and the strains in yeast extract and mannitol broth during 5 and 10 days. two mL of rhizobial suspension (individually) were applied at sowing time and six days later. The plants were watered and harvested 45 days after planting. Nodule number and dry weight, shoot nitrogen concentration and content, shoot and root dry matter, and total dry weight were evaluated. Rhizobia inoculation was significant for all parameters evaluated. The highest shoot nitrogen content and total dry biomass identified the strains JV91, JV94, and JV101 as effective. Shoot nitrogen content correlated significantly with shoot dry weight ($r = 0.85$), total dry weight ($r = 0.90$), and nodule biomass ($r = 0.72$).

P0115. The leaf pathogen *Cymadothea trifolii* locally degrades pectins, but not cellulose or xyloglucan in clover cell walls.

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The biotrophic pathogen *Cymadothea trifolii* proliferates intercellularly within leaves of *Trifolium* species. The fungus produces a unique structure, which we have termed interaction apparatus (IA), in its own hyphae. Opposite the IA, the plasmalemma of the host cell invaginates to form a bubble. The two structures are linked by a 300 nm wide tube leading through the walls of plant and fungus without disrupting them. A wide array of antibodies against different plant cell wall components and a fungal polygalacturonase were applied to high-pressure frozen, freeze-substituted and Epon, HM20 or LR White embedded leaves of heavily infected *Trifolium repens* plants. Samples were studied with the electron microscope. Our results show that within the tube pectins are degraded, while cellulose and xyloglucan are left intact. Furthermore, a polygalacturonase was detected in functioning but not in old IAs. The degradation of the pectin matrix probably results in an increase of pore diameter in the plant wall at the point of interaction.

P0116. Artificial bacterial symbiosis as a tool for extending biological nitrogen fixation to asymbiotic plants.

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Artificial symbiotic associations were established between aerobic nitrogen fixing *Azotobacter* species and different plants from unicellular green algae to perennial crops with the methods of cell and tissue culture and regeneration. The description and further utilization of such systems required the incorporation of molecular

markers (first GUS and later wild-type and mutant forms of GFP) into symbiont candidate bacteria to detect and monitor them inside and outside their host cells and tissues. The performance of different transformation methods was compared using different plasmids. The techniques adapted for the preparation of transformation competent bacterial cells and electroporation provided significantly more viable transformants than the formerly used procedures. Chromosomal integration of transforming plasmids and a stable expression of marker genes were also observed. The new strains grew on carbohydrate and nitrogen free media having normal morphological and growth characteristics. Bacterial nitrogenase retained its activity in inoculated or regenerated plant material. This work was supported by OTKA T-034875 grant.

P0117. Are grapevine moving particles from *Vitis rupestris* responsible for the resistance to powdery mildew (*Uncinula necator*)?

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Both the American grapes (*Vitis* spp.) and the European one (*V. vinifera*) are important for the viticulture. The American grapes are usually resistant to powdery mildew, whereas the European is more susceptible. Grapevine moving particles (GMPs) were first observed around 20 years ago. The number of GMPs per cell was found to be directly correlated with the plant resistance to *Uncinula necator*. The cellular number of GMPs in the American grape was 15 to 30 times higher than in the European. There is a clear increment in the number of GMPs during an infection process. GMPs exhibit random movement *in vitro* and *in planta*. The movement of isolated GMPs was reduced in an ATP-depleting system. Purified GMPs are round to ellipsoid, with a size ranging from 100 to 400 nm, and contained xylanase, carboxymethyl cellulase, chitinase activities and other proteins. A serine/threonine-specific protein kinase was also identified, an enzyme possibly required for GMP movement. GMPs strongly inhibited powdery mildew spore germination, appressorium formation and penetration. These results suggest that GMPs are at least in part involved in the grapevine defence system.

P0118. Histopathological changes in plant tissues due to fungal infection

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The interaction between a host and pathogen has been divided into three consecutive phase which although interdependent are physically separated by the host surface, namely, growth of the pathogen prior to penetration, penetration, and growth within the host. The growth of the pathogen within the host and the histological changes in plant tissues had been studied in different hosts with special references to cowpea and cucumber plants. *Rhizoctonia*, *Phoma*, *Macrophomina* & downy mildew fungi caused important diseases to the tested plants and different histopathological and ultrastructure changes were observed and photomicrography using bright field and scanning electron microscope. The most important changes recorded were changes in plant cell color and size, increased of cambial activity, location of strange particles within tissues, deposition of callose in xylem tissues, death of cells, increase in the number of vacuoles, distortion of cells, tissues collapsed, and many other changes. Most of the previous mentioned changes were photographed and examined in different plant organs & seeds.

P0119. Effects of PGPR on the Growth of Maize (*Zea Mays* L.)

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In the framework of the soil increased pollution with chemical fertilizers, the maintaining and growing of the crops impose finding

alternative solutions based on biofertilizers usage. One group of microorganisms which are beneficial to crops is bacteria that colonize roots or rhizosphere soil of crop plants which are referred to as plant growth promoting rhizobacteria (PGPR).

Several PGPR strains influences (isolated from *Zea mays* rhizosphere) were studied on the maize plants development in field conditions (a bio-preparation with a concentration of 16.4×10^7 CFU/ml was used).

The results indicate a stimulation of growing and development processes (the catalase activity recorded in the test was double compared to the control; the content of photosynthetic pigments was higher in the leaves of the test plants; biometrical parameters and the harvest were significantly higher on the test). The PGPR beneficial influence was recorded before flowering when majority metabolic processes intensity is highest, probably, because of stimulation radicular nutrients exchange mediated by rhizobacteria.

P0119a. Direct RT-PCR amplification of mature mRNAs in cytoplasm micropipetted from barley coleoptile epidermal cell - A model system for analyzing gene expression in host cells attacked by powdery mildew

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Two-step PCRs (RT-PCR and nested PCR) were used to detect gene expression in powdery mildew pathogen-infected cells of detached inner epidermis of barley coleoptile. Cellular contents of the infected cells were microscopically suctioned with a micropipette and subjected to the PCRs. Two genes involved in the glycolytic pathway and stimulus-induced endochitinase gene were targeted, and their expression was determined by detecting cDNAs derived from spliced transcripts. Two glycolysis-related genes were constantly expressed in the tissue irrespective of pathogen inoculation. Contrarily, the chitinase gene expression was induced in non-infected inner epidermis after the detachment. After inoculation, this expression was selectively suppressed in pathogen-invaded cells, in spite of continuous expression in non-invaded cells of the same epidermis. Thus, the present method enabled us to directly analyze transcripts in individual cells at the infection site and indicate the capability of the pathogen to regulate host gene expression.

P0120. Epigenetic transposon regulation in Arabidopsis neopolyploids

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Polyploids are common and arise frequently by genome duplication (autopolyploids) or interspecific hybridization (allopolyploids). In spite of their frequency in nature, early generations of human-made allopolyploids often display sterility, inviability, phenotypic instability, gene silencing, gene activation, and epigenetic changes. We have previously shown that several transposons, including the novel CACTA-like transposon *Sunfish*, are transcriptionally and, less frequently, transpositionally activated in neopolyploid offspring of synthetic crosses of *Arabidopsis thaliana* and *A. arenosa*. Transposons may thus play an important role in the evolution of novel traits in neopolyploids. We have assessed *Sunfish* activity in diploid chromatin mutants of *A. thaliana* and in several accessions of the natural allopolyploid *A. suecica*. Our data correlate *Sunfish* activity both with the loss of epigenetic control in *A. thaliana* and with polyploidization in the natural allopolyploid *A. suecica*.

P0121. Expression of ribosomal RNA genes in two Tragopogon allotetraploids of recent and recurrent origin

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Nucleolar dominance is an epigenetic phenomenon where one parental set of ribosomal RNA (rRNA) genes is silenced in interspecific hybrids. We investigated silencing of parental rRNA genes in multiple populations of *Tragopogon mirus* (*T. porrifolius* x

T. dubius) and *T. miscellus* (*T. pratensis* x *T. dubius*), allotetraploids formed within the last century. Natural variations in rRNA gene silencing were found among individual populations. In most populations, rRNA transcripts of *T. dubius* origin outnumbered those of the partner genome. Since rDNA units of *T. dubius*-type are partially lost through homogenization, it is likely that genetic events leading to reduction of one parental type units did not prevent their expression and dominance. In one population of *T. mirus* the general trend of silencing was reversed and *T. porrifolius* units were predominantly expressed showing that bidirectional rRNA silencing within a single species can occur. In this population silencing was unstable and expression patterns varied even among progeny of a single plant. Leaf and flower expression patterns were similar suggesting that silencing is not influenced by development.

P0122. Expression profiling and polyploidization in *Gossypium*

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The reunification of two divergent genomes via hybridization and subsequent doubling (allopolyploidy) requires instantaneous coordination of the two suddenly co-resident genomes. Although allopolyploidy may be accompanied by some level of genome rearrangement, little is known about regulatory interactions and gene expression changes. Previous work in *Gossypium* has shown that many genes experience expression alteration on a tissue-specific basis. Using synthetically derived cotton (*Gossypium spp.*) allopolyploids, we explored the effect of polyploidization on patterns of gene expression for approximately 12,000 loci using 70mer oligonucleotide technology. Using controlled growth conditions in a replicated block design, we examined expression alteration in two independent synthetic polyploidy events, including one involving diploids representing the genome donors of the economically important *G. hirsutum* and *G. barbadense*. Here we present the analysis of these experiments and the evolutionary implications for recently formed polyploids.

P0123. The effects of colchicines and the induction of polyploidy in sorghum bicolor (Keller, IS4546 and R10 cultivars).

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This investigation was set out to determine the most suitable treatment for inducing polyploidy in Sweet sorghum using colchicines. Three methods of colchicines application for three cultivars are used, namely (1) to seed, (2) to apical meristem of seedlings and (3) to seedlings. In these methods colchicines concentration was 0-0.5% for 4-72 hours. The percent of mortality for three cultivar (Keller, IS4546 and R10) was 89.7 to 98.9%. The normal chromosome number in three cultivars was $2n=2x=20$. In 0.75% to 12.2% pollen mother cells (pmc) of these cultivars were induced by colchicines, chromosome number was double or $2n=4x=40$ (in Is4546= 12.2% and in Keller= 0.75%). In cytogenetically studies beside autotetraploid, lagging and no disjunction was observed too. The lagging phenomenon for these cultivars was high (15-28%). Also aberration such as fusion of two or more pmc's with 30, 40 and 52 bivalent chromosomes (5, 7 and 2%) was observed.

P0124. Meiotic elaboration of the of homeologous pairing suppressing genes on genomes B and C of Brassica

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Meiotic analyses of 8 digenomic triploids, 4 each having genomic combination of AAC and AAB, synthesized through hybridizing *Brassica rapa*, *B. juncea* and *B. napus*; showed gross meiotic anomalies in the form of univalents and multivalents. The rate of chromosomal associations, orientation of multivalents and combination types was different from genotype to genotype. The hybrids showed differences in the frequencies of associating chromosomes at the individual levels but the percentage of paired and unpaired chromosomes remained nearly equal in both types of the digenomic triploids. The ratio of paired and unpaired chromosomes was c 27 and 26 percent in the having genomic

constitution ABA and ACA, respectively. The average ratio of the pairing and non pairing chromosomes remained 70 to 30 percent, respectively. The high frequency and nearly equal amount of IIs in both the digenomic triploids revealed the absence of gene on genomes B or C, which could be held responsible for a role of suppressing homeologous pairing in Brassica.

P0125. Differential expression analysis of aberrant and normal phenotypes in *Brassica oleracea*

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Phenotypic aberrations affecting leaves morphology and vigor during the vegetative development of plants have been described in all the observed varieties of *Brassica oleracea* var. *botrytis*. In field conditions, 3 to 20% of plants can show these aberrations, the percentage varying according to both plant genotype and environment. The phenomenon can be reversible, suggesting the involvement of epigenetic mechanisms.

In this study, we investigate the expression polymorphism between "normal" and "aberrant" individuals by using two complementary approaches: cDNA-AFLP and candidate genes. Candidate genes were chosen given their roles in transcription regulation, epigenetic controls or in leaf morphology.

Differential expression was analyzed at 6 kinetic points corresponding to different developmental stages in the F1 hybrid Nominoé. Forty bands were differentially expressed in either normal or aberrant plants, at different kinetic points, notably when the aberrant phenotype appeared. Thirty of them have been sequenced. Results will be discussed in regard of epigenetic mechanisms described in *Arabidopsis thaliana*.

P0126. Patterns of homeologous recombination in Brassica napus haploids

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Interspecific hybridization and polyploidy are major forces driving the evolution of plants. Structural rearrangements, sequence divergence and gene(s) are known to modify the rate of homeologous recombination and thus the rate of gene flow between related species/genomes. Here, we present the analysis of homeologous recombination in *Brassica napus* haploids (AC, $n=19$). *B. napus* (AACC, $2n=38$) is an allotetraploid whose progenitors are *B. oleracea* (CC, $2n=18$) and *B. rapa* (AA, $2n=20$). We have analyzed the progenies of high and low pairing haploids and used the non transmission of alleles from the haploid parents as an evidence for homeologous recombination. We show that the gene PrBn has a major effect on the level of homeologous recombination; we provide evidence that the amount of homeologous recombination depends on the level of synteny between the A and C recombining chromosomes. These results will be discussed with regards to the emergence of allopolyploids.

P0127. Secondary Thickening by MRI in Stem of Eucalyptus grandis

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MRI of Hydrogen nuclei at low field strength was used in imaging of a plant of *Eucalyptus grandis* to study secondary thickening of its stem as it was taking place. Low field strength was chosen so as to minimize artefacts at air water interfaces. The plant grew with supplementary illumination and regular watering and an occasional commercial minerals solution was given to prevent mineral deficiency.

The plant was vigorous and added several branches during the experimental period.

Two months during which secondary growth was visualised show the method's power

The same plant was killed after the experiment and its structure imaged by a Leica stereozoom optical microscopy.

We identify the same tissues in both imaging systems. However the pith shows stratification by MRI into an outer and inner

zone. MRI of studies of secondary thickening permit dynamic studies of this process in living plants without the destructive methods of classical plant anatomy.

P0128. Spatial and temporal relation between cytokinin localisation and ectopic expression of a class1 knotted like gene in peach leaves affected by the curl disease caused by *Taphrina deformans*

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In plants, the class1 knotted-like transcription factors (KNOX) are crucial in determining the identity and fate of meristem cells. The down regulation KNOX1 genes is necessary for leaf primordia initiation, while their overexpression leads to alterations of plant architecture and organ morphology

A KNOX1 gene of peach (KNOPE1) was isolated and attributed to class 1 based on its specific expression in the SAM and on its aminoacidic identity. KNOPE1 transcript was localised in leaves affected by the curl disease, caused by *Taphrina deformans*, a fungus able to produce auxin and cytokinin-like compounds. The disease is characterised by hypertrophic and hyperplastic development of leaf accompanied by a misbalance and/or misallocation of phytohormones. In situ analysis was performed on leaves at distinct phases of the disease, revealed that KNOPE1 message was abundant and misplaced as compared to healthy leaves, and zeatin (Z) highly accumulated in curly tissues. Moreover, in sectors of histological disorder, the localization (Z) overlapped that of KNOPE1 transcript suggesting a spatial and a temporal relationship between gene regulation and (Z) accumulation

P0129. Improvements of shoot meristem identity and somatic embryo development of white spruce (*Picea glauca*) through modulations of glutathione metabolism.

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Optimizing propagation of tree species through somatic embryogenesis remains one of the main objectives in sustaining superior quality genotypes for re-forestation strategies. In white spruce, improvements of embryo development were observed following experimental alterations of the endogenous glutathione redox state. An increase in embryo number and quality can be obtained through initial applications of reduced glutathione (GSH), which promote cell proliferation and the formation of immature embryos, followed by applications of the oxidized form (GSSG), which induce proper embryonic development. Structural and ultrastructural studies reveal marked differences between the meristematic cells of the control embryos and those of the treated counterparts. In addition, RNA in-situ localization studies of a shoot apical meristem marker gene, HBK1 (Sundas Larsson et al. 1998, P.N.A.S. 9: 1118) indicate that changes in glutathione metabolism during embryo development improve shoot apical meristem stability, and ultimately regeneration of viable plants.

P0130. Function Analysis of the Millet (*Setaria italica*) Gene *PF40* Involved in Plant Apical Dominance

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The aerial architecture of flowering plants is determined to a large extent by shoot growth and shoot branching arising from the initiation and growth of axillary meristems. But the underlying regulatory mechanisms have not been unraveled. *PF40* gene isolated from the cDNA library of millet immature seeds encodes a 30KDa protein with 35%-75% sequence identity to the ZIPs gene family. Software analysis showed *PF40* protein had eight transmembrane regions. Southern blot indicated that *PF40* gene was ubiquitous distributed in cereal crop genome. Northern blot showed *PF40* gene was expressed in all tissue of millet but predominant in very young tissue such as hypocotyl and bud and the expression level can be regulated by 6-BA and GA. Transgenic plants showed that overexpression of *PF40* gene can reduced the apical dominance of both millet and tobacco by effecting the growth of lateral shoots developing in the basipetal pattern early after floral transition. The ratio of auxin/cytokinin was more than 5 fold in the *PF40* gained function plant than that in wild type.

Furthermore the *PF40* gene effected differentiation of the vascular bundles in transgenic plants.

P0131. Characterization of novel systemic non-coding small RNA molecules isolated from pumpkin phloem sap

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Plant growth and tissue differentiation is coordinated via exchange of local and long distance signals. Regulatory signals in form of micro RNAs, which inhibit the translation of mRNA can move cell-to-cell and throughout the plant using the long distance phloem pathway. These mobile micro RNAs are suggested to control pattern formation and cell fate. It was shown that non-cell-autonomous micro RNA and mRNA present in the phloem sap are transported by RNA-binding proteins via plasmodesmata to gain access to the phloem translocation pathway. Interestingly, in the phloem sap of pumpkin we could detect additional RNA molecules not resembling mRNA or micro RNA with unknown function. These novel non-coding RNA molecules change in size and number during tissue maturation and by environmental impacts. We started to isolate and characterize these RNA molecules to gain insights into their potential function. Currently we are establishing cDNA libraries representing these systemic small RNA molecules with the aim to predict potential target genes and to study their putative function(s) in systemic signaling.

P0132. Highly frequent RNA editing in *rbcl* gene of Takakiophyta

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The genus *Takakia* is sometimes treated as independent division. We analyzed the partial nucleotide sequence of the chloroplast gene *rbcl* (ca. 1260 bp) from *T. lepidozoides* from Japan, Taiwan, and Malaysia, and *T. ceratophylla* from China. There was no difference between each sequences of *T. lepidozoides*. There were twenty one differences between the sequence of two species. Thirteen differences of them were correspond to non-synonymous substitutions, in which twelve were between T and C. Many C to U substitutions were especially found as the result of RNA editing, which had been reported in the chloroplast genomes of some plants. We analyzed the cDNA sequence of *rbcl* gene from *T. lepidozoides* from Taiwan with RT-PCR. There were thirty two differences between the genomic and cDNA sequences. We confirmed that the RNA editing were frequently occurred in the *rbcl* gene of *Takakia*. Twenty four sites of thirty two were considered as editing sites common to both species. *T. ceratophylla* seems to have additional eight editing sites independently.

P0133. Efficiency of RNA isolation from potato protoplasts

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Resynthesis of cell wall after protoplasts isolation is far of being known closely. Potato protoplasts were used as source for RNA. Potato cultivars were tested for protoplast isolation efficiency and were chosen for the RNA experiment. The RNA probes were meant to be a part of a microarray experiment, which should give an idea about the cell wall regeneration in the first days after the isolation of protoplasts. To get a general idea about the regeneration process the protoplasts were stained with different reagents (Calcofluor White, Yarov reagent, Aniline Blue) and with some antibodies for plant cell walls were used. For the extraction of RNA we made several tests for a method that uses lithium chloride and we also tested for extraction a kit (RNeasy Plant Mini Kit). The RNA which was isolated using the kit was qualitatively superior to the one using the LiCl. After comparing the results from the staining experiments were chosen 5 time points to make the experiment. First time point at 5 hours from isolation and then at 12h, 24h, 36h, 48h. For each time point an amount of more than 29 µg of total RNA was obtained-which was considered sufficient for a microarray experiment.

P0134. A NAP (nucleosome assembly protein)-like polypeptide binds specifically chloroplast intron RNAs of *Chlamydomonas reinhardtii*

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In *Chlamydomonas reinhardtii* the chloroplast *psaA* gene is fragmented into three independently transcribed exons which are flanked by consensus sequences of group II introns. To generate a mature *psaA* mRNA two *trans*-splicing steps are necessary involving a small chloroplast-encoded RNA (*tscA*). In recent years, the *C. reinhardtii* system has been used to identify components which are part of a postulated chloroplast spliceosome that is involved in the *trans*-splicing process.

In our effort to isolate further factors binding specifically intron RNAs, we used the yeast three-hybrid system to identify chloroplast RNA binding proteins. By using the *tscA* RNA as bait, we identify a clone encoding a 40 kD prey protein with similarity to nuclear localized nucleosome assembly proteins (NAP). In contrast to nuclear NAPs, the derived polypeptide carries a 44 aa chloroplast signal. Therefore the corresponding gene was designated *Nap-like*. The organellar localization of NAPL was shown with an antibody which detected specifically a recombinant NAPL-Tag fusion protein. Thus, NAPL is most probably part of a chloroplast spliceosome.

P0135. DYW domain proteins and RNA editing: a phylogenetic correlation

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PPR (pentatricopeptide repeat) proteins form a huge family (about 450 members in *Arabidopsis*) defined by repetitions of characteristic motifs. Genetic and functional data support that these proteins are implicated as specificity adaptors in post-transcriptional processes in plant organelles, including RNA editing. The proteins catalysing RNA editing are currently unknown. We suggest that the DYW C-terminal domain present in many PPR proteins could be the essential enzyme for this process. The sequence of this domain contains conserved residues that match the active site of cytidine dealinases. A study of the phylogenetic distribution of this domain in green plants, in particular in liverworts of which one class (the Jungermaniidae) edit their RNA, while the other (the Marchantiidae) do not, was performed. Our results suggest a correlation between the presence of the DYW domain and RNA editing in green plants.

P0136. Characterization of suppressor of wooden leg 1: a mutant showing impaired vascular development

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The *Arabidopsis* *wooden leg* (*wol*) mutant root has a reduced number of provascular cell files and all cell files differentiate as xylem during vascular morphogenesis resulting in determinate root growth pattern (Scheres et al. 1995). *WOL* is allelic with *CRE1/AHK4* and encodes a cytokinin receptor (Mähönen et al. 2000, Inoue et al 2001, Suzuki et al 2001). In order to identify molecules acting downstream of CRE1, we performed a suppressor screen for the determinate growth habit of *wol*. Here we show the characterisation of one of these suppressor mutants, *suppressor of wol1* (*sow1*). In the *wol* background, *sow1* shows an increased number of vascular cell files in the root-hypocotyl junction with undifferentiated files present, as opposed to the exclusively protoxylem cell files present in *wol*. Alone, *sow1* shows defects in maintaining the balance between cell division and differentiation during root vascular morphogenesis.

P0137. Ca²⁺-mediated signalling and programmed cell death induced in soybean cells by an endopolygalacturonase from *Sclerotinia sclerotiorum*

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Sclerotinia sclerotiorum is a ubiquitous necrotrophic pathogen secreting multiple pectinolytic enzymes that facilitate penetration, colonization and maceration of plant tissues. Among these enzymes, various isoforms of endo-polygalacturonases (PGs) are produced. A basic PG, early expressed in *S. sclerotiorum* during soybean infection, was used to analyse cellular responses in soybean cell cultures. PG treatment triggered a rapid increase in the concentration of cytosolic Ca²⁺ which was maintained over the basal level during the next hour. Loss of cell viability in the same time interval was observed, with a progressive increase in the percentage of dead cells over 12h. PG-induced cell death was associated with cytochrome *c* release from mitochondria and caspase-like protease activation, indicating that a programmed cell death (PCD) pathway was triggered. As demonstrated by the effect of the Ca²⁺ channel blocker La³⁺, the increase of cytosolic Ca²⁺ level seems to be essential for the induction of PCD by PG. When a polygalacturonase inhibiting protein (PGIP) and the PG were simultaneously applied to cells, both Ca²⁺ increase and cell death were annulled.

P0138. Programmed cell death is induced by mild heat stress in soybean cells via a mitochondrial-mediated pathway

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Programmed cell death (PCD) is fundamental for normal growth and development of multicellular organisms and in response to environmental stresses. The pathway of PCD has been partially clarified in plants although the underlying molecular mechanisms are still poorly defined. We have investigated the signalling cascade induced by a mild heat treatment causing PCD in soybean cells. The data show that heat shock led to the onset of PCD in soybean cells involving H₂O₂ production and mitochondrial damage. Cytochrome *c* release is followed by the activation of the death-related caspase 9-like and caspase 3-like proteases. Concomitantly, cells were severely damaged with a progressive cell shrinkage, chloroplast alteration and detachment of the plasma membrane from the cell wall. Chromatin condensation and DNA laddering formation were observed. It is concluded that a mild heat stress induces PCD in soybean cells through a caspase-dependent mitochondrial pathway.

P0139. Stress-induced programmed cell death in the unicellular alga "*Chlorella*" *saccharophila*-like

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Programmed cell death (PCD) processes have been reported extensively in animal and plant cells under different environmental stresses. However, the key elements of stress-induced PCD remain largely unresolved in unicellular organisms. In this study, the occurrence of PCD-like cell death was determined in the unicellular chlorophyte "*Chlorella*" *saccharophila*-like using specific indicators of plant and animal PCD. Our results show that, under heat and salt stresses, cells undergo a form of cell death with morphological features of apoptotic cell death, including an increase in chromatin condensation and margination, nuclei shrinkage and DNA fragmentation. Assays on the activity of caspase 3-like enzyme, a cell death-associated protease, showed an increase with the treatment and paralleled the morphological changes. Moreover, the caspase-like activity was inhibited by specific caspase inhibitors. The finding of a stress-induced caspase-mediated cell death pathway together with the occurrence of key components of eukaryotes PCD in a chlorophyte, suggest the hypothesis that key elements of cell death pathways were conserved during evolution.

P0140. Effect on the changes of antioxidant to chilling in *indica* and *japonica* subspecies under chilling temperature (*Oryza sativa* L.)

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Six rice varieties namely *Japonica* Taibei 309, Wuyujing, *indica*R64, Pusa, CA212 and *indica* Hybrid Shanyou 63 were used to study the effect by chilling temperature (8°C). The light intensity-photosynthesis curves, CO₂-photosynthesis curves, primarily photochemical chemical efficiency, active oxygen species, glutathione and ascorbate content in their seedlings were measured before and after chilling treatment. The results showed: relative to the rice varieties tolerant to chilling such as Taipei 309

and Wuyujing, the sensitive ones IR64, Pusa, CA212 exhibited a stronger inhibition of Maximum photosynthetic rate and a decrease of Fv/Fm, which led to the accumulation of AOS. It was also found that the content of glutathione disulphide in glutathione pool and that of dehydroascorbate in ascorbate pool of the leaves of these sensitive ones under chilling were induced to increase obviously. The correlation coefficient among the increases of GSSG, DHA and the decrease of Chl content, Chl content and ASA/DHA, GSH/GSSG were -0.701**, -0.656** ,0.811**and 0.728** respectively, significant at 0.01 probability levels.

P0141. Active transposon *Tok* in rice

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Rice is a major crop and a model plant for grass family. Recent completion of the rice genome sequencing revealed that rice genome contains about 16% transposon. But most of the transposable elements are inactive. In this report, we identified an active transposable element *delta-Tok*. *Delta-Tok* was inserted at the kinase domain of *MP2* that is homologue of *CLAVATA1*, causing multiple pistil mutant phenotype. *Delta-Tok* elements are members of the *Ds*-like transposable elements and short 664 bp long. *Tok* elements are about 3.5-4.0 kb long and contain an open reading frame encoding a putative transposase. Database search of *Nipponbare* identified 25 copies for *delta-Tok* and 9 copies for *Tok*, sharing terminal inverted repeat and subterminal domains. Our data suggest that *delta-Tok* and *Tok* are active transposable elements ubiquously present in *Oryza* genus. These elements should have played important roles during evolution and they may be used for insertional mutagenesis of rice.

P0142. Regulation of tobacco CDKA kinase activity by phosphorylation.

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We investigated the regulation of CDKA-associated kinase activity in tobacco BY-2 cells that had been partially synchronized by transferring stationary cells into fresh medium. We found that kinase activity of p13^{SUC1}-bound proteins, which contain cyclin/CDKA complexes, increased significantly at the G1/S phase. Western analysis revealed that CDKA protein gradually accumulated during cell cycle re-entry, indicating that changes in protein levels were at least partially responsible for the elevated kinase activity. To determine whether post-translational modification of CDKA was also involved in modulating kinase activity, immunoblots were probed with phosphorylation-specific antibodies to either Tyr-15- or Thr-161-phosphorylated human Cdc2, the homolog of CDKA. While phosphorylation of Thr-161 increased rapidly during cell cycle re-entry, no phosphorylation of Tyr-15 was detected. These results suggest that CAK (CDK-activating kinase) activates CDKA kinase at the G1/S phase by phosphorylating Thr-161.

P0143. An E2F-Regulated reporter construct is transcriptionally activated following the transient expression of cyclin D in plants.

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The E2F transcription factors play important roles in the regulation of gene expression in plants. Here, we show that the rice proliferation cell nuclear antigen (PCNA) promoter is activated by transient expression of tobacco NtE2F and Arabidopsis AtDPA in tobacco BY-2 cells. This transcriptional activation is repressed by co-transfection with a plasmid encoding the tobacco Rb-related protein (NtRBR1), whereas further co-expression of cyclin D overcomes this repression. Importantly, the rice PCNA promoter is activated when cells are transfected with cyclin D alone, and this activation is enhanced by co-transfection with plasmids encoding

NtE2F and AtDPA. These results suggest that the effect of cyclin D expression is mediated not only by its associated kinase, which allows it to phosphorylate NtRBR1 thereby releasing the NtE2F/NtDP complex to activate transcription, but also by a mechanism which does not involve transfected NtRBR1.

P0144. Functional Analysis of CDK Inhibitors from *Arabidopsis Thaliana*

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Seven Kip-related protein genes (*KRP1-7*) encode *Arabidopsis* CDK inhibitors showing restricted similarities with an animal p27^{KIP1}. Here, we analyzed the functions of KRP proteins. To ascertain the significance of the C-terminal conserved region, we constructed deletion derivatives. Kinase-inhibition activity was lost in only 16 amino acids C-terminal deletion, whereas all tested N-terminal deletions retained the activity. Additionally, mutated proteins in KYNF/YD motif significantly reduced inhibition activity. These results suggested that inhibition of Cyclin/CDK was attributable to the C-terminal region, and KYNF/YD motif was essential. In binding assays, KRP1-7 proteins interacted with CyclinD2/CDKA complex but not with Cyclin D2 and CDKA protein alone. In kinase assays, GST-tagged KRP proteins inhibited kinase activity of Cyclin D2/CDKA and Cyclin D2/CDKB complexes in a different dose-dependent manner. Interestingly, GST-KRP1 and -7 proteins inhibited kinase activity of Cyclin D2/CDKA higher than that of Cyclin D2/CDKB. We will discuss the relationship between inhibition activity and binding ability to Cyclin/CDK complex in KRPs.

P0145. Protoplast culture and plant regeneration of the methionine resistant plantlets of *Astragalus melilotoides* Pall.

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An efficient protocol for plant regeneration from protoplasts of the methionine resistant variant of *Astragalus melilotoides* was established. The friable calli induced from internode segments of variant plants were used for protoplast preparation. The protoplasts were isolated through enzyme digestion. Calli were formed after sustained divisions of protoplasts. High frequency of shoot differentiation was obtained from the protocalli on differentiated medium. The effects of different media, culturing methods and plating densities on protoplast divisions and plant regeneration were studied. The results show that agarose-beads culture method, KM8p medium supplemented with 1.0mg/L 2,4-D, 0.5mg/L 6BA, 0.3mol/L mannitol, 2%(W/V)sucrose and 500mg/L casein hydrolysate at a plating density of 3×10^5 /mL are the appropriate conditions for protoplast division of the methionine resistant cell line. The division frequency is over 38%. The protoplast-regenerated plants still preserve resistance to methionine and ethionine. This research builds up the foundation for the resistant cell line as a parent of somatic hybridization.

P0146. Effects of *Chelidonium majus* L. extracts on the process of plants cells-division

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In this research we used ethanol and aqueous extracts of the root and shoot parts of *Chelidonium majus* L. Meristematic cells of *Allium cepa*'s young roots were treated by these extracts. Then the root tips were cut and fixed in glacial acetic acid: ethanol (1:3) for 24h and preserved in 70% ethanol for cytological preparation used of squash technique. 500 cells were analyzed for cell division and chromosome segregation results analyzed statically methods. Our results showed all cells treated 48-72 h by extracts were killed degenerated.

In cells that treated 24h, most of cells stopped in G2/M and couldn't complete their cell cycles and start cell division. Effects of root extracts were more than shoot extracts and effects of aqueous extracts were more than ethanol extract. All cell death phenomena were taken by Apoptosis. In third group (that treated by extracts for 4h) more cells stopped in Metaphase and the number of cells that stopped in G2/M were little. In control specimen all stages of a cell

cycle were abundant and these cells continue their cycle which any block wasn't Sean.

P0147. Impact of ectomycorrhiza formation on monosaccharide transporter gene expression in poplar roots

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Most trees of temperate and boreal forests form ectomycorrhizas, a symbiosis of plant fine roots with certain soil fungi. The essence of this interaction is the exchange of plant-derived carbohydrates for fungus-derived mineral nutrients.

Five monosaccharide transporter genes were isolated from poplar ectomycorrhizas by RT-PCR. The three most strongly expressed genes are up-regulated in symbiosis. The strongest effect was obtained with *PttMST3.1* (ten-times higher expression rate in non-infected roots compared to other genes), that revealed a further twelve-fold up-regulation by ectomycorrhiza formation. This strong increase of *PttMST3.1* expression in mycorrhizas, but not that of the other genes, requires an active plant/fungus interaction.

The up-regulation of hexose transporter genes by mycorrhiza formation suggests, that root cells are able to compete with fungal hyphae for hexoses from the common apoplast during symbiosis. A hypothesis how the plant can coordinate sugar-efflux towards the fungal partner with a fungus-derived support with mineral nutrients at a local level will be presented.

P0148. Mutagenic Effects of Gamma Radiation in Mungbean (*Vigna radiata* (L) Wilczek)

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In the present investigation an attempt was made to induce mutations in mungbean (*Vigna radiata* (L) Wilczek) Variety Vaibhav, employing gamma radiation with an objective of obtaining novel and desirable mutants. Seeds of mungbean were irradiated with 30, 40 and 50 kR doses of gamma radiation and sown in the experimental field to raise M1, M2 and M3 populations. Mutations were screened at three generations. All the three doses of gamma radiations were effective in producing various morphological mutations. But the 50 kR dose of gamma radiation was effective in producing a novel mutant that showed multiple morphological mutations like large sized flowers with dark yellow petals, dense thick hairy pods and black coloured seeds. They are named as lhb mutant (Large flowers, hairy pods and black seed mutants). Important morphological characters of lhb mutants are described and discussed in the text.

P0149. Towards a better understanding of nuclear mechanisms involved in legume seed development

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The quality of legume seeds is progressively established during seed development and the major seed proteins are known. However, the regulatory pathways controlling the expression levels of proteins related to seed development (e.g. storage proteins) are largely unknown and need to be investigated as a basis for improving legume seed quality traits. The aim of this work is to identify nuclear genes (e.g. transcription factors; modulators of chromatin structure) expressed at defined stages of seed filling in the model legume *Medicago truncatula*. These are under characterization by microarray-based analysis of nuclear gene expression and by affinity chromatography of nuclei enriched fractions followed by one- and two-dimensional electrophoresis. The identity of nuclear proteins will be determined from 2D gel fractionated spots by Quadrupole-Time Of Flight Mass Spectrometry and from protein extracts or 1D gel fractionated bands by nanoscale Liquid Chromatography tandem MS. The role of these nuclear genes in the control of seed development will be assessed by screening the TILLING collection in *M. truncatula* for mutants at the corresponding genetic loci.

P0150. Integrating New Knowledge into the rms Model of Branch Inhibition in Pea.

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The study of shoot branching has primarily been the study of apical dominance and auxin inhibition. New mutants in pea (*ramosus*) and Arabidopsis (*more axillary branching*) are extending the signals involved in the control of this phenomenon. The anatomy and branching habit of pea makes it a practical model for physiological studies and the presence of an orthologous set of mutants in Arabidopsis allows for the cloning of these genes in pea using PCR techniques. The cloning of the *RMS* genes are providing evidence for new levels of regulation in the *rms* model and helping us to further elucidate this pathway of crosstalk that controls branching.

The *rms1* through *rms5* mutations increase both basal and aerial branching. This phenotype can be almost completely rescued by grafting a WT rootstock to *rms1*, *rms2* or *rms5* mutant shoots. The cloning and characterization of *RMS1*, *RMS4* and *RMS5* has provided us with new tools to test our hypotheses relating to IAA and IAA-independent regulation of branching.

P0151. Effect of Some Materials on *Sesamia cretica* Infesting Some Maize and Sorghum Varieties

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Effect of carbamate insecticide Methomyl, bacterial insecticide Agreïn, Petroleum oil, Sisi6 and inorganic salt, Barium nitrate against the first and third larval instar of *S. cretica* collected from infested maize plants in the field. Results indicated that methomyl was the most toxic compound compared with the rest. LC50 values were 1.06x10⁻², 1.18x10⁻⁴, 1.58x10⁻⁴ and 2.23x10⁻⁴ ppm for Methomyl, Sisi6, Barium nitrate and Agreïn, respectively. Effects of the same compounds against *S. cretica* on some maize and sorghum varieties were also tested in the field during 2002 and 2003 seasons. Results indicated that Methomyl was the most effective material in reducing the infestation representing 89.73, 92.92, 83.29 and 92.30 % reduction during 2002 season on hybrid single 10, hybrid single 3080, hybrid third 313 and balady varieties of maize, respectively. The mean of reduction % in 2003 for Sisi6 and Barium nitrate were less than 46% in all tested varieties of sorghum. Factors affected reduction percentages of *S. cretica* and the recommended material for controlling the pest were discussed.

P0152. Transcriptional activation of tobacco E2F is repressed by co-transfection with the retinoblastoma-related protein

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We have characterized NtE2F by using the tobacco PCNA promoter containing two E2F binding sites. Each E2F element conferred a different degree of contribution to the transcriptional activation, but mutation of both E2F elements greatly reduced the activation ability of the reporter gene at G1/S phase in synchronized tobacco BY-2 cells. Furthermore, in transient assays effective activation of the tobacco PCNA promoter was observed by co-transfection with the plasmids containing NtE2F and the newly identified tobacco NtDP. However, this transactivation is repressed by co-transfection with a plasmid encoding NtRBR1, and this repressor activity of NtRBR1 was cancelled when it was further co-transfected with a plasmid containing cyclin D but not with cyclin A or cyclin B. Our results suggest that two E2F binding sites in tobacco PCNA promoter are synergistically responsible for transcriptional activation at G1/S phase, and that, like mammalian cells, transfected cyclin D-associated kinase complexes probably phosphorylate NtRBR1, thereby releasing the E2F/DP complex to activate transcription.

P0153. Effects of P deficiency on cell production and expansion

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Leaf expansion is severely inhibited by P deficiency, which can be alleviated by symbiosis with arbuscular mycorrhizal fungi (AMF). We tested whether leaf elongation rate (LER, mm h⁻¹) and its components: cell production rate (cell h⁻¹) and final epidermal cell length (mm cell⁻¹) are related to P concentration in the leaf growth zone of *Lolium perenne*, and whether these relationships are modified by AMF *Glomus hoi*. P concentration in the growth zone increased with increasing P supply and presence of AMF. LER was linearly related to P concentration in the growth zone and no P status-independent effects of AMF on LER were observed. P deficiency did not affect significantly meristematic cell size, and cells spent a longer time in the cell elongation zone due to a decreased displacement velocity. However, substantially reduced relative cell elongation rates resulted in a decreased final cell size in P deficient plants. Further, P deficiency decreased cell production rate, presumably due to lower cell division rates in the intercalary meristem. In conclusion, both cell production and cell expansion parameters were related to the P status of the growth zone.

P0154. The effect of cadmium and salicylic acid on some physiological parameters of Brassica napus

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Abstract:

In this investigation we studied the effect of cadmium and salicylic acid on some physiological phenomena of *Brassica napus* L. In pot plants we measured the contents of Chl a, Chl b, carotene and Xanthophyll contents in shoots and cadmium, proline and the activity rate of catalase and peroxidase enzymes and different growth parameters in shoot and root of treated plants. The lower concentration of SA increased germination. On the other hand, the fresh weight of 10 days seedlings decreased with increasing of SA and Cd but the length of stem and fresh weight of 10 days seedlings increased at 50 µM SA. In pot plants with addition of 100µM SA decreasing of dry weight, Chl a, b, carotene, xanthophyll and proline contents were shown. In 10 days seedlings, with increasing of Cd, the Chl a contents decreased. With increasing of cadmium in soil, the cadmium contents in shoot and root of treated plants increased. Increasing of SA caused increasing of peroxidase activity in root but decreasing of this enzyme activity in shoot.

P0155. Promoter analysis of a JA- and ethylene-independent but wound-responsive tobacco peroxidase gene, *tpoxN1*

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Plants respond to mechanical wounding inducing accumulation of defense-related proteins including class III peroxidase (POX, EC 1.11.1.7). We isolated a tobacco POX gene, *tpoxN1*, whose expression is induced not only by infection of tobacco mosaic virus but also by wounding (Hiraga et al. 2000). Interestingly, *tpoxN1* transcript predominantly accumulated especially in stems within 1 h after wounding. But the induced expression was not affected by JA and ethylene (Sasaki et al. 2002), which are well known wound signals. Here, we show that wound-responsive cis-element of *tpoxN1* was present in the 49 bp at the proximal promoter region using transgenic plants carrying the *GUS* reporter genes driven by deletion series of the *tpoxN1* promoter. To the 49-bp fragment in *tpoxN1* promoter, wound-inducible nuclear factor(s) bound in a sequence specific manner in gel mobility shift assay. Competitive gel retardation assays indicated that the nuclear factor(s) bound 11-bp element in the 49-bp, which is also conserved in the distal region of the *tpoxN1* promoter. These results indicated that the 11-

bp region would be important for the wound response of *tpoxN1* gene.

P0156. Physiological leaf spot in barley as a response to light stress.

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Physiological leaf spot (PLS) is a disorder of spring barley (*Hordeum vulgare* L.), which has become more pronounced in recent years. The initial symptoms are small chlorotic/brown spots on the upper four leaves, which may develop into necrotic lesions with an irregular shape. The symptoms do not necessarily occur every year and also vary in intensity between cultivars. A definitive aetiology for this disease has not yet emerged but it is suspected to have an abiotic origin as no microorganisms have been cultured from affected leaves. As PLS occurs on leaves that are directly exposed to sunlight it is thought high light stress could be a trigger for the condition. The study concentrates on two cultivars, Cooper and Crusader, which display differential sensitivity to PLS. A substantial difference in levels of ascorbate, type III peroxidases, and superoxide dismutase between the chosen cultivars was confirmed for the growing seasons 2003 and 2004. Current work is focused on looking at gene expression related to these biochemical findings.

P0157. The Effects of Salt Stress and Paclobutrazol on Some Morphological Parameters in Sensitive and Resistant Cultivars of Wheat

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The effect of salt stress and Paclobutrazol (PBZ) treatments in the two cultivars of wheat (resistant and sensitive to salt stress) were studied in this research. Wheat plants were irrigated with 0, 75, 150 and 225 mmol / lit NaCl solutions and sprayed with 0, 30, 60 and 90 ppm Paclobutrazol then some morphological parameters were analyzed. Studied showed that in the sensitive cultivar salt stress caused reduction in fresh and dry shoot, root and sixth leaf weight, and shoot water content. While Paclobutrazol caused reduction in sixth leaf length and area, shoot length, special sixth leaf area and weight. We observed that Paclobutrazol increased fresh weight of shoot, root and sixth leaf weight, and water content of shoot and sixth leaf. Therefore we concluded that Paclobutrazol treatment can moderate the effect of salt stress on morphological parameters in sensitive cultivar of wheat.

P0158. Stress induced emission of biogenic VOC's - their temporal behaviour and the relation of VOC emissions from different biosynthetic pathways

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Exposing plants (*Nicotiana tabacum* var. Bel W3, *Lycopersicon esculentum* var. Moneymaker, *Gossypium Hirsutum* var. Stoneville) to ozone induced VOC emission bursts. Ozone stress led to temporally increased emissions of methanol, VOC from the octadecanoid pathway (LOX products), methyl salicylate (MeSA) and sesquiterpenes. Aim of our work is to test whether or not good correlations exist between the stress induced emissions and plant internal precursor / signal molecules. Such good correlations would imply usage of gas phase data as a non-invasive tool for studying the activity and dynamics of biosynthetic pathways as response to abiotic or biotic stresses. Using a computer assisted tool to develop or optimize HPLC methods for complicated separation (CHROMWORD HPLC software) we optimized the HPLC method. A lot of plant internal compounds were found to be increased in their concentrations after ozone stress. As a first result of our measurements we observed a good relationship between the content of salicylic acid in the plant and the emission of methyl salicylate.

P0159. Differential gene expression of transporters, the Halliwell-Asada pathway and iron homeostasis implicates Zn/Cd accumulation and tolerance in *Arabidopsis halleri*

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For survival, plants possess remarkable homeostatic mechanisms to maintain a suitable concentration of essential heavy metal ions in vivo. Certain plants, which accumulate very high levels of heavy metals have been named hyperaccumulators. By using Arabidopsis cDNA microarray, we compared the gene expression profiles of the hyperaccumulator, Arabidopsis halleri (ssp. halleri and gemmifera) with a non-hyperaccumulator, Arabidopsis thaliana. We demonstrate that differential regulation of genes involved in metal transport, translocation and antioxidation are associated the metal hyperaccumulation capacity in Arabidopsis halleri. Also, the activity of hydrogen peroxidases in Arabidopsis halleri was much higher than that in A. thaliana. The high peroxidase activity in A. halleri is also observed. This implicates the capacity to detoxify H₂O₂ produced in cadmium and paraquat treatments. Interestingly, Fe feedback regulation was observed in A. halleri.

P0160. LZF1, a light-regulated zinc finger transcription factor, acts downstream of HY5 in Arabidopsis

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Global gene expression interrogation via DNA microarray has revealed genes with distinct or common patterns in photomorphogenesis. Our microarray data revealed that LZF1, a light- and HY5-dependent zinc finger protein, is up-regulated in response to light signal in Arabidopsis. Failed to be up-regulated in hy5 mutant plants, LZF1 apparently functions downstream of the bZIP transcription factor HY5. LZF1 is distally related to a group of more well-characterized transcription factors, the CONSTANS (CO) family. When the gene expression dosage of LZF1 is ectopically increased in Arabidopsis, the developmental program is severely affected by showing retarded growth and late flowering phenotypes. The accumulation of LZF1 protein exhibits a light-stable and dark-labile manner. LZF1-GFP protein is localized in the nucleus, which implies LZF1 functions inside the nucleus. Putative target genes of LZF1 are revealed by comparing the gene expression profiles of Arabidopsis cells with and without the overexpression of LZF1 proteins.

P0161. Investigating the role of a family of bHLH transcription factors in determining root epidermis cell fate.

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A major challenge in developmental biology is to understand the molecular basis of the specification and patterning of different cell types in multicellular organisms. In plants, the formation of the hair and non-hair cells in the Arabidopsis root epidermis is used as a simple model for studying cell fate specification. We have taken a genetic approach to investigate the molecular mechanisms that specifies cell fate in the root epidermis. We identified a family of basic Helix-Loop-Helix (bHLH) transcription factors acting at a late stage of the hair cell differentiation process. These include *ROOT HAIR DEFECTIVE 6* (*RHD6*) and four *RHD6-LIKE* (*RDL1*, *2*, *3* and *4*) proteins. My project is to understand how these transcription factors act in root hair development. Specifically, I will test the ability of *RHD6* and *RDL* proteins to activate transcription, to form dimers, to be targeted to the nucleus and to bind DNA. I will use different approaches including yeast one- and two-hybrid systems, immunological techniques, DNaseI footprinting, microscopy and genetics.

P0162. Effectiveness of Phragmites australis plants in removing phosphates from a phosphate enriched aqueous solution and its relationship with photosynthetic metabolites

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Phytoremediation is the use of plants to remove pollutants and toxic materials from both soils and water. Excessive amounts of inorganic phosphorus in water can cause significant water quality problems, provoking reproduction and expansion in big quantities of algae and plants (eutrophication). Constructed wetlands for wastewater treatment are increasing all over the world, using aquatic plants as wastewater purifiers. On the other hand

Phosphorus is an essential nutrient for plants that use it in different processes as photosynthesis.

In this study we have grown *Phragmites australis* plants under three different nutritional conditions: being supplied with 1 mg/l, 10 mg/l and 100 mg/l KH₂PO₄. A foliar analysis has been carried out to determine plant's ability to assimilate this phosphate.

The total phosphate content in the leaves have been determined as well as the concentration of some photosynthetic metabolites as phosphoglyceric acid, pyruvate, ADP, ATP, glucose-6-phosphate. Preliminary results indicate that there is a direct relationship between the KH₂PO₄ levels in loads and both phosphate content in leaves and their photosynthesis.

P0163. Bioindication and biomonitoring studies carried out in rural and in urban sites by ozone sensitive clover clones (Trifolium repens cv Regal).

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This study has been carried out in the summer of 2001/04s by using O₃ sensitive white clover clones to estimate the O₃ effects on biomass and on frequency of leaf damage in natural, sub-urban and urban areas of Rome. All periods showed a Mediterranean climate having high values of temperature and solar radiation and scarce precipitations. This climate determined high values of O₃ concentrations with hourly average peaks up to 129 ppb, and an increase of O₃-induced leaf injury was also due to the establishing of limiting environmental conditions. The entity of leaf damage, however, turns out to be different in the areas. In urban and sub-urban sites, it was induced from the interaction of more factors, while in the rural ones it could be caused directly from O₃, taking into consideration also biogenic and anthropogenic volatile organic compounds. Temperature represents an important factor determining the entity of O₃-induced leaf injury on clover. Moreover, high atmospheric pollutants and temperature values along a linear gradient lasting coast to Rome urban area created a complex system that could mask the real O₃ effects on plants.

P0164. Arabidopsis gene URO promote the auxin response genes without increasing the concentration of free IAA

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The phytohormone auxin occupies a central place within plants' signaling transduction network. But we know little about the detail of the network. *uro* is an Arabidopsis semi-dominant mutant with some phenotype that always connected with auxin defaults. We found that there are strong epistasis *DR-5* expression in whole young seedling and mature stem of *uro* mutant. At the same time, there are no evidence shows that the level of free IAA in *uro* is higher than in wild type, through IAA immunolocalisation test. *URO* gene promotes the auxin response genes without increasing the concentration of free IAA. *URO* gene may play an important role in connect auxin signal transduction pathway and the other signaling pathway. We cloned *URO* gene. it is an transcription factor with a C2H2 zinc finger domain. There is few homologous gene in other creatures besides plants. It is possible that the *URO* gene is a gene which carrying out a proper function of plants.

P0165. Studies for the conservation of Coccothrinax crinita ssp. crinita: an endangered Cuban palm.

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The specie *Coccothrinax crinita* (Griseb. et Wendl. ex Wright) Becc. ssp. *crinita* is a palm endemic to Cuba. It is critically of threatened and appears in the list of species of most threatened palms of the insular territories of the Caribbean; because of this it is a priority for studying the conservation of this taxon. The goal of this research is to carry out a compare studies between natural population and *ex situ* population at the National Botanical Garden of Cuba to develop conservation programs. The studies were carried out in the collection of the Botanical Garden and in natural population. Important data were taken on the number of individuals, accompanying vegetation, risk factors and interference agents. Mature fruits were collected from both populations to carry out the germination assay and viability of the seeds. The chromosome number was determined by treatment of root tips.

Samples of healthy leaves were also collected for the study of genetic diversity by ISSR-PCR techniques. This results are very important for the conservation ex situ and in situ of *Coccothrinax crinita*. We to propose a new endangered category in relation with IUCN.

P0166. Cytokinin Signaling in Secondary Vascular Development

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The secondary phase of plant development is initiated through the activation of a lateral meristem, the vascular cambium. Secondary vascular tissues are produced via cell divisions taking place in the cambium. The Arabidopsis genome encodes a cytokinin receptor gene family with three members (*CRE1/WOL/AHK4*, *AHK2*, *AHK3*). In a triple mutant lacking all three receptor genes, both the size and activity of apical meristems are reduced, consistent with the idea that cytokinins are positive regulators of meristem activity (Higuchi et al. 2004 PNAS USA 101: 8821-6).

We have investigated the role of cytokinin signaling in the secondary vascular development in two tree species, silver birch and poplar. We have identified from birch three (*BpHK1-3*), and from poplar five (*PtHK1-5*), genes which are highly homologous for the three Arabidopsis cytokinin receptor genes. We have shown that the *BpHK* genes are active in the cambial zone, and that the putative birch ortholog of CRE1, BpHK1, is functional as a cytokinin receptor in Arabidopsis. We are currently producing transgenic trees in which the cytokinin signaling is downregulated in the cambial zone.

P0167. Regulation of ion channels by ABC proteins in guard cells: Over-expression of AtMRP5 in tobacco increases drought sensitivity and decreases slow-anion channel activity.

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Guard cells in the epidermis are responsible for the gas exchange and transpiration regulation. In Arabidopsis, AtMRP5 which is an ABC transporter belong to the multi-drug resistance protein (MRP) subfamily, expressed in guard cells. Knock-out mutants are more resistant to drought stress and stomata movement was insensitive to ABA and auxin. MRPs transport organic compounds using ATP, but are also candidates for modulating other membrane proteins, such as ion channels. To study in detail the mechanism of stomata modulation by AtMRP5, we over-expressed AtMRP5 in the *Nicotiana tabacum*. At both plant and leaf level, over-expression of AtMRP5 led to an enhanced transpiration rate. In AtMRP5 over-expression lines, stomata opened more in light and ABA dependent closure was reduced. Patch clamp technique was applied to test if ion channel activity is affected by over-expression of AtMRP5 in guard cells. K⁺ outward current was slightly smaller in comparison to the wild type. In addition, the activity of slow anion channel was smaller in the AtMRP5 over-expressed plants. These results suggest that AtMRP5 contribute to the stomata opening by modulation of ion channels.

P0168. Development of microsatellite markers for assessing genetic diversity of alpine *Rhododendron* varieties

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Rhododendron hirsutum and *R.ferrugineum* are *Rhododendron* species endemic to alpine areas in Austria, Slovenia, Italy and Switzerland. The species is of ecological and economical interest, as, due to its natural habitat it can be used as plant cover for skiing slopes, where it prevents erosion. It is also of interest for gardeners for being planted in rock gardens.

In order to assess the existing genetic diversity of the species in Austria, we were developing a SSR marker system. We constructed an enriched library from *Rhododendron* spp. From this

library, 176 clones were sequenced, but only 27 inserts showed microsatellite sequences of considerable length. The other 149 sequences only contained short repeats.

25 sequences were used for primer design. These primers were tested for variability on a restricted set of *Rhododendron* accessions. 17 of the 25 designed primers revealed variable fragments in the chosen test set, but only 11 of these regions were producing a nice clear banding pattern and could be used for further analysis.

P0169. Protection of *Medicago truncatula* against *Fusarium acuminatum* and defense gene expression profiling in response to an elicitor from *Paenibacillus* sp. strain B2

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ABSTRACT

The ability of a plant growth-promoting rhizobacterium to induce plant resistance against a biotic stress was studied. An antagonistic lipo-polypeptide (paenimyxin factor) from *Paenibacillus* sp. strain B2 was used to elicit defence related responses of the model legume *Medicago truncatula*. For this purpose, a pathosystem between *M. truncatula* and *Fusarium acuminatum* was established. Plant roots were incubated with different concentrations of the paenimyxin factor for 24 hrs prior to inoculation with *F. acuminatum*. At a concentration of 1 µM, paenimyxin had a protective effect, suppressing 95% of the morphological infection symptoms, whereas, the factor had an inhibitory effect on plant growth at a concentration higher than 10 µM. Plant gene expression was quantified by semiquantitative RT-PCR. Genes involved in the biosynthesis of phytoalexins (PAL, CHS and CHR), coding proteins having an antifungal activity (pathogenesis-related proteins and chitinase) or cell wall invertase were highly upregulated after the paenimyxin treatment. The mechanism involved in plant protection by the paenimyxin factor against *F. acuminatum* are discussed.

P0170. Early events in the molecular interaction between the biocontrol agent *Trichoderma harzianum* and soybean cells

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Trichoderma spp. are commercially applied as biopesticides to control a variety of plant pathogens. The effects of *Trichoderma* colonization on plants include an improvement of plant growth and metabolism and the induction of systemic and localized resistance to phytopathogenic fungi, bacteria and viruses. We investigated the early events occurring during the interaction between *Trichoderma harzianum* and cultured soybean cells, by applying to cells the culture media of this fungus, grown alone or in direct antagonism with the necrotrophic pathogen *Botrytis cinerea*. The results indicate that molecules released in the fungal culture media are perceived by plant cells as elicitors and activate a Ca²⁺-mediated signalling. Specific responses such as intracellular H₂O₂ accumulation and programmed cell death involving caspase-like proteases are triggered downstream the Ca²⁺ signal. Both Ca²⁺ signature and cell responses are modified by the addition to cells of the culture medium from a knock-out mutant of *Trichoderma*, defective for the production of a 42-kDa endochitinase.

P0171. Resistance to potato late blight detected in a wild potato genetic resource, *Solanum caripense*: preliminary results

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Resistance to *Phytophthora infestans*, causing late blight, exists in the gene pool of wild *Solanaceae*. However, only a small number of these have been used so far for introgression of resistance (R) genes into cultivated potato, *Solanum tuberosum*, leaving late blight a big constraint all over the world. We are locating genes for resistance to *P. infestans* in *S. caripense*, a wild, non-tuber-bearing relative of potato and tomato. On a biparental population of 206 siblings, AFLP and SSR primers were used to obtain 360 markers for construction of a genetic linkage map. The markers

mapped on eleven linkage groups for the maternal, and all twelve for the paternal parent. Resistance tests with four *P. infestans* isolates revealed high intensities of sporulation on susceptible plants regardless of lesion size, suggesting that the resistance in question may directly be sporulation-related. The maps will be used to locate genetic markers associated with resistance and enable gene isolation and cloning.

P0172. Comparative expression profiling of rice near isogenic lines reveals genes related to bacterial blight and fungal blast resistance

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Comparative cDNA microarray analysis was performed to study the possible correlation between gene expression profiles and rice disease resistance related phenotypes. Near isogenic lines carrying different *Xa* (resistant to bacterial blight) or *Pi* (resistant to fungal blast) genes, and their susceptible parents were analyzed in parallel to minimize differences due to variation in genetic backgrounds. Clustering analysis showed that different disease resistance related phenotypes exhibited distinct gene expression profiles, providing evidence to support the notion that global transcriptional re-programming is a major component of signal transduction leading to disease resistance. Classification analysis was also adopted in this study and successfully identified reporter clones with high prediction power. Using the combined advantages of unique genetic materials collected and the power of cDNA microarray technology, we have set up the prototype of a research platform that can be used to perform comparative analysis of disease resistance related phenotypes based on gene expression profiling, and to identify important genes in due course.

P0173. Contribution of target transgene position and structure to RNA-directed promoter methylation and TGS

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RNA-directed transcriptional gene silencing (TGS), the repression of transcription of a gene in correlation with promoter DNA methylation in the presence of double stranded (ds) RNA with homology to the respective promoter, has been studied extensively in plant transgene systems employing the nopaline synthase promoter (NOSpro) in *Arabidopsis thaliana* and tobacco. Not all target transgenes show the same susceptibility to RNA-directed TGS, indicating that the chromosomal location of target transgenes and / or the particular arrangement of NOSpro copies in the target transgenes might contribute to the silencing process. Well-characterized target transgenes with the same structure integrated at different chromosomal positions or with differing structures integrated at the same chromosomal positions are presently being challenged by a silencer transgene providing NOSpro dsRNA in *Arabidopsis thaliana*. Current results of the analysis of induced transcriptional repression and NOSpro DNA methylation will be presented. The work is supported by DFG grant SFB 648 TP C4.

P0174. Centromere breakage and gene silencing induced by T-DNA insertion in *Arabidopsis thaliana*

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In addition to two aberrant chromosomes, two types of minichromosomes were generated by T-DNA insertion into the major centromeric satellite of chromosome 2 in *Arabidopsis thaliana*. FISH (fluorescence *in situ* hybridization) with 5S and 18S rDNAs and BAC clones as probes clearly revealed that both of the minichromosomes have originated from the short arm of chromosome 2 (2S). One minichromosome (mini2S-A) contained the whole short arm, but the size of the satellite (180-bp repeat) cluster was much shorter (<1 Mb) than that of the original chromosome 2. The inserted T-DNA was found at the distal end of the satellite that has been healed with telomeric DNA. This suggests that the T-DNA insertion induced the centromere

dysfunction and breakage. Interestingly, expression of both *GFP* and *nptII* genes on the inserted T-DNA were suppressed in some of the transformed plants, but not in others. The state of the transgenes is now being analyzed, but this sort of gene silencing must be induced by chromatin and DNA modifications between the centromeric and *de novo* telomeric regions.

P0175. The Mobility of a Non-TIR Mutator-like DNA Transposon in Arabidopsis

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Most Mutator-like elements (MULEs) are characterized by terminal inverted-repeats (TIRs) and the generation of a target site duplication (TSD) upon insertion. However, in the *Arabidopsis* and other plant genomes, some MULEs appear to lack TIRs but have the same characteristic TSD length and the MULE-specific transposase gene, *mudrA*. We show that a DNA transposon called non-TIR MULEs in *Arabidopsis*, acquired a host gene encoding a polypeptide with a conserved peptidase-C48 domain characteristic of ULP-like host genes. In order to confirm whether these MULEs are active, a transposon display technology was employed to map any new insertion or excision event in wildtype (ecotype Columbia-0) and in mutant backgrounds known to activate mobile elements; namely *ddm1*, *met1*, *cmt3* and a *met1/cmt3* double mutant. From this survey one member from one MULE family was found to be mobile and only in the *cmt3/met1* double mutant background. This is the first indication of non-TIR MULE mobility.

P0176. Chlorophyll a fluorescence proves that growth-promoting endophytes alleviate Cd stress in *Cicer arietinum* Linn.

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Chl a is used as rapid and non-destructive biomarker for the assessment of stress caused by different environmental factors. Cd stress was induced in *C. arietinum* (Chick-pea), which is the third important crop of India. Stress of Cd causes chlorosis and necrosis to the plants.

The chl a measurements were taken after 10 days of inoculation with the help of Handy PEA. Cd stress was given to each plant after 14 days from sowing, excluding control (without stress) of each variety. The concentration of Cd was increased after a period of 5 days by 10 µg to increase stress. We used *Piriformospora indica*, *Glomus caledonium* and *Glomus mosseae* as inocula to evaluate effect of Cd stress on *C. arietinum*. The observations were recorded in Handy PEA at the regular interval of 7 days up to 30 days. But when Cd conc. was increased to 100 µg, observations were recorded on each 4th day. The fluorescence rise was analyzed by 'Biolyzer' program.

The main aim of the present study was to assess the efficiency of endophytes on *C. arietinum* exposed to Cd stress.

P0177. Direct Shoot Organogenesis for *Aster ericoides* cultivar "Monte Cassino"

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Direct organogenesis was induced from "in vitro" leaf explants of *Aster ericoides* cultivar "Monte Cassino" cultured on MS (1962) medium supplemented with 6-benzilaminopurine (BA) (2.5 mM, 4.5 mM), *n*-naphthaleneacetic acid (NAA) (2.5 mM, 1 mM), indole-3-acetic acid (IAA) (17.1 mM) and gibberellic acid (GA) (14.43 mM) and then solidified with agar (6 g l⁻¹) or gelrite (2 g l⁻¹). After 30 days, the best response to direct shoot induction was observed on MS medium supplemented with BA 2.5 mM and NAA 2.5 mM. In this medium was got an average of 4 shoots (10%) after 45 days of culture. On MS medium supplemented with GA 14.43 mM and BA 2.5 mM solidified with agar, the shoots also developed directly from explants (7 %), but they were hyperhydrated. The highest number of shoots (17% and 33%, respectively) was obtained on MS medium supplemented with BA 2.5 mM and NAA 1 mM and solidified either with agar or gelrite, but they developed from callus tissue. These results show that direct organogenesis is obtained on MS medium with BA and ANA. Also, direct organogenesis is obtained on MS medium solidified either with agar or gelrite, but the gelrite contributes to shoots vitrification.

P0178. Boundaries and phyllotaxy in Arabidopsis

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In plants the arrangement of organs around the stem (phyllotaxy) follows a predicted pattern.

We studied phyllotaxy in Arabidopsis WS ecotype and observed variability in the phyllotaxy pattern. Then we characterised mutants -pny/blr, caf1/dcl1 and hyl1- presenting altered phyllotaxy: clusters of primordia and random divergence angle between successive primordia. All three mutants present a common feature: enlarged boundaries characterised by wider CUC1 and 2 expression domains. Partial complementation of the abnormal phyllotaxy in hyl1 and pny was obtained by reducing the boundary domain. Furthermore phenocopies of the mutant were obtained by inducing the enlargement of the boundary domain in wild-type plants.

These results suggest the existence of a feedback mechanism linking the boundary domain with the process of phyllotaxy control. As auxin has been shown crucial for phyllotaxy, we are currently investigating the involvement of auxin in this mechanism.

P0179. The planktonic and benthic organisms of Lake Van

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In the present study, the identification of approximately 70 taxa were identified. Most of them are phytoplankton. The specimen were collected from Lake Van between 1990 and 1995. The specimen were identified under light microscope and photographed. The identification of specimen were made in species, genus and/or family.

Water samples were collected from different altitude in selected 18 stations of Van Lake between 1990-1995 collected and identified planctons and their density are given on tables. The highest number of genus or species are following:

1. *Oscillatoria limosa*
2. *O. tenuis*
3. *Anabaena affinis*
4. *Spirulina major*
5. *Microcystis* sp.
6. *Closterium* sp.
7. *Cymatopleura* sp.
8. *Navicula* sp.
9. *Synedra* sp.
10. *Cyclotella* sp.
11. *Gleocapsa* sp.
12. *Anchistrodesmus* sp.
13. *Pinnularia* sp.
14. *Colurella* sp.

The preparates of samples has been performed and their photograpies were taken.

P0180. Characterization of candidate transcriptional regulators of stem development and fiber differentiation in Arabidopsis thaliana

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Stages of vascular and interfascicular fiber differentiation can be identified along the axis of bolting stems in Arabidopsis. To gain insights into the regulatory events that control this pattern, we used transcript profiling employing an Arabidopsis full genome longmer microarray to assay global changes in gene transcription over the course of stem development and fiber differentiation. Among 5000 differentially expressed genes, we identified 182 up-regulated transcription factors (TF) that represent candidate regulators of fiber development. A subset of these TF candidates has been associated with fiber development and/or secondary wall formation in other studies. These candidates include members of the MYB, bHLH, AP-EREBP, KNAT, and C3H families. T-DNA knockout lines of most candidates have been identified and are under analysis to determine if and how these genes control fiber differentiation. We have also identified putative orthologues of many of the candidate TF in the completed poplar genome sequence. Investigation of these genes will allow greater insight into their potential roles in secondary wall and wood formation in this model tree.

P0181. Target specificity of RNA silencing in transgenic plants with coat protein gene of Sweet potato feathery mottle virus

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We have previously reported the graft transmission of the target specificity of RNA silencing in transgenic *Nicotiana benthamiana* plants with the coat protein (CP) gene of *Sweet potato feathery mottle virus*. To further characterize its transmission, we first investigated a silenced line of 4.09, of which the target specificity is the 3' 400 bp of the transgene. Northern blot analysis showed a RNA band (700 bp) in this line shorter than that (1,200bp) of a high expressor (4.11). Further analysis with different probes showed that the RNA band hybridized with the 5' 400 bp but not with the 3' 400 bp of the CP gene. Secondly transgenic silenced lines with 5' part of the CP gene were produced and grafted onto 4.09 rootstocks. No changes of RNA banding patterns in scions and rootstocks were observed, indicating no interactions of silencing signals between the two different silenced lines. Grafting of 4.09 scions onto the silenced scions with the 5' part is in progress to see any further degradation of RNA from the 5' part of the transgene in 4.09. The results will be presented and discussed in relation to the transmission of target specificity for RNA degradation.

P0182. Phospholipase C from Physcomitrella patens: physiology and knockout

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In eukaryotes phosphoinositide-specific phospholipase C (PI-PLC) plays an important role in various signal transduction pathways. Plant PI-PLCs are part of signalling osmotic stress, gravity- and light perception. The moss *Physcomitrella patens* contains at least five genes encoding PI-PLCs, four of which are sequenced. Analysis of the amino acid sequences revealed two separate groups (PpPLC1 and 4, PpPLC2 and 3). One member of each group was characterised enzymatically (PLC1 and PLC2), showing that they belong to biochemically different PLCs (Mikami et al. 2004).

We generated plc1, plc2 and plc3 gene-targeted knockouts via homologous recombination in *Physcomitrella*. The loss of PpPLC1 results in developmental defects implying a role in cytokinin signalling, gravitropism and chloroplast development (Repp et al. 2004). The knockout of PpPLC2 has no obvious phenotype under standard growth conditions. However, the treatment with stress mediating substances like abscisic acid or mannitol, indicated that PpPLC2 is involved in ABA-dependent stress signalling. The physiological role of PpPLC3 is currently under investigation and will be discussed.

P0183. Effect of three AM fungi on growth, distribution of glandular hairs and essential oil in Ocimum basilicum L. var. Genovese

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The essential oils of basil are widely used in the cosmetic, pharmaceutical, food and flavouring industries. Little is known about the potential of arbuscular mycorrhizal (AM) fungi to affect the production of essential oil in aromatic plants. The effects of colonization by three AM fungi *Glomus mosseae* BEG 12, *Gigaspora margarita* BEG 34 and *Gigaspora rosea* BEG 9 on shoot and root biomass, abundance of glandular hairs and essential oil yield of *Ocimum basilicum* L. var. Genovese were studied. The AM fungi induced various modifications of the considered parameters, but only *Gi. rosea* significantly affected all of them in comparison with control plants or the other fungal treatments. It significantly increased biomass, root branching and length, and the total amount of essential oil (especially camphor and -terpineol). Increased oil yield was associated to a significantly larger number of peltate glandular trichomes (main sites of essential oil synthesis) in the basal and central leaf zones. Results showed that different fungi can induce different effects on the same plant and that the essential oil yield can be modulated according to the colonizing AM fungus.

P0184. Arbuscular mycorrhizae in non-mycotrophic plant species

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Arbuscular mycorrhizal (AM) symbiosis is supposed to be the most widespread mutualistic relationship in plant kingdom, however, members of some plant families do not normally form mycorrhizae. Non-mycotrophic plant species are the first colonisers of newly established man-made ecosystems, which lack AM fungi. Interaction of AM fungi with non-mycotrophic species initiating primary succession on coal-mine spoil banks (*Atriplex sagittata*, Chenopodiaceae and *Sisymbrium loeselii*, Brassicaceae) was studied in two microcosm experiments. These plants were inoculated either 1) with spores, fragments of extraradical mycelium (ERM) and colonised root pieces (the way commonly used in most experimental studies) or 2) via the network of ERM pre-established on a mycotrophic plant. The results show that both non-mycotrophic plant species can be colonised via the ERM network and form fully developed mycorrhizal structures including arbuscules. Temporal development of intraradical colonisation and effect of AM on plant growth differed between plant and fungal species.

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P0185. Plastid reorganization in root cortical cells colonized by arbuscular mycorrhizal fungi

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Plastids are reacting to the colonization of root cortical cells by arbuscular mycorrhizal (AM) fungi by (i) a marked proliferation leading to network-like organelle structures and (ii) the biosynthesis of AM-specific apocarotenoids of unknown functions. The combination of metabolite and transcript profiling revealed that apart from apocarotenoids, plastids produce higher amounts of fatty acids and amino acids in AM roots. These compounds are apparently necessary for formation of the symbiotic interface, explaining the marked multiplication of lens-shaped plastids close to young arbuscules. Apocarotenoid formation, in contrast, is activated during the later phase of arbuscule degradation. The tubular plastids formed then are in part decorated by rings of the plastid division protein FtsZ and thus possibly involved in repeated fusion/division events. Plastid metabolism, in this phase, is possibly involved in recycling degraded fungal material. Formation of apocarotenoids during arbuscule degradation might be connected to the concomitant accumulation of hydrogen peroxide.

P0186. Gene expression in arbuscular mycorrhizal fungi

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Arbuscular mycorrhiza (AM) is a mutualistic symbiosis between roots of most vascular land plants and obligate biotrophic fungi of the phylum Glomeromycota. In order to elucidate the molecular basis of the interaction, we are studying gene expression patterns. Three projects will be presented which are focussed on the identification and characterization of fungal genes. Differential display analysis indicated that fungal respiratory pathway genes are induced by root exudates in the presymbiotic phase of *Gigaspora rosea*. These results were confirmed by oxygraphy and staining of mitochondria. Numerous genes were identified in extraradical hyphae of *Glomus intraradices* during the symbiotic phase by suppressive subtractive hybridization, which are suggested to be involved in biofertilization and bioprotection. A family of GST encoding genes could be identified which were induced by heavy metals. A third project deals with the intraradical hyphae and a number of genes involved in C metabolism of the fungus have been characterized by Real Time PCR.

P0187. Mitochondrial large subunit rDNA sequences as novel marker genes in the Glomeromycota

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Partial sequences of the large subunit of mitochondrial rDNA (mtLSU) were obtained from the arbuscular mycorrhizal fungi *Glomus proliferum* and *Glomus intraradices* (Glomeromycota) cultivated in root organ cultures. Three isolates of *G. intraradices* were analyzed. There was no evidence of intra-isolate sequence heterogeneity as it is found in variable regions of nuclear ribosomal genes in the Glomeromycota.

In *G. intraradices*, but not in *G. proliferum*, two introns were found in the partial mtLSU sequence. One of them contained an ORF for a putative site-specific endonuclease of the LAGLIDADG family. Phylogenetic analysis of the endonuclease sequence showed its closest known relatives to be hosted in other fungal mitochondrial introns.

The apparent absence of intra-individual variation and the gene structure of alternating short conserved and variable regions with strong length variation make the mtLSU valuable as a future marker gene for molecular identification purposes. The intronic sequences may be useful as an even more sensitive molecular marker for phylogeography and population studies.

P0188. Root exudates of mycorrhizal tomato plants exhibit a different effect on *Fusarium oxysporum* f. sp. *lycopersici* than root exudates from non-mycorrhizal tomato plants

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Compounds in root exudates are important signals in plant-microbe interactions. Thus, changes in the root exudation pattern can affect the outcome of plant-microbe interactions.

Root colonization by arbuscular mycorrhizal fungi changes the root exudation pattern of plants. Root exudates of mycorrhizal plants have been shown to exhibit a different effect on the hyphal growth of AM fungi, on sporulation of *Phytophthora* species and chemotropism of bacteria and zoospores, than root exudates from non-mycorrhizal plants.

We found that root exudates of mycorrhizal tomato plants differently affect the spore germination of *Fusarium oxysporum* f. sp. *lycopersici* than root exudates from non-mycorrhizal tomato plants. This effect did not depend on the improved P-status of the mycorrhizal tomato plant. A first characterization of the bioactive compounds was performed.

The impact of alterations of the root exudation pattern in mycorrhizal plants on soil microorganisms is discussed.

P0189. Enhanced Hexose Supply in Tobacco Roots has no Effect on Mycorrhization

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In the mutualistic association of arbuscular mycorrhiza (AM), the fungus assists the plant with the acquisition of mineral nutrients from the soil, whereas the plant supplies the fungus with carbohydrates. Sucrose is most likely hydrolyzed by apoplastic invertases and hexoses are then taken up by intraradical fungal structures.

To analyze the role of invertases in the mycorrhizal association, we used transgenic tobacco (*Nicotiana tabacum*) plants expressing an apoplast-located yeast-derived invertase under the control of the inducible *alc* promoter system derived from *Aspergillus nidulans*. After root-specific induction of the invertase, an enhanced invertase activity and an increase in the ratio of hexoses to sucrose was found. Upon inoculation with *Glomus intraradices*, however, the altered sugar levels did not show any effect on the mycorrhization degree and the formation of fungal structures. Moreover, the contents of AM-specific free fatty acids and fungus-induced cyclohexenones remained unchanged.

P0190. The effect of mycorrhization on the production of essential oils in herbal plants

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In the production of essential oil crops three parameters are of importance, the composition of the essential oil, the content of the essential oil in the plant and the essential oil yield as the product of content and the production of biomass. Arbuscular mycorrhizal fungi (AMF) do not only affect plant growth, but also the

accumulation of secondary plant compounds from different chemical groups. We tested the effect of an arbuscular mycorrhizal fungus on yield and essential oil composition of two economic plants, oregano and sage, both used as medicinal as well as aromatic plants. Plants were inoculated with AMF (*Glomus mosseae*) and kept in the greenhouse and fertilized with a standard nutrient solution without phosphorus (control group and AMF group) and a standard mix with phosphorous to be able to compare any effect with the effect of phosphorous fertilization. The shoot dry weight, the P-concentration in the shoot, the degree of root colonization by the AMF and the qualitative and quantitative content of essential oils in leaves were determined.

P0191. Influence of pesticides on arbuscular mycorrhiza of selected *Populus* clones and soil microbial community structure

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Selected *Populus* species and hybrids may have a high potential for phytoremediation of soils and groundwater contaminated by pesticides. As poplars in nature form both, ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) symbioses, the tolerance of mycorrhizal fungi and associated bacteria to the xenobiotics is one of key condition in application programs. In this study four poplar clones, inoculated by indigenous mycorrhizal fungi and by and introduced AM isolate (*Glomus intraradices*, BEG 87) were grown in soil highly contaminated by pesticide substrate, originated from a pesticide tomb in Poland. The degree of colonisation of roots by mycorrhizal fungi, estimated both, by microscopic observation and by signature lipid fatty acids analyses indicated inhibition ECM development and significant decreased of AM colonisation by indigenous mycorrhizal fungi in contaminated soils. The introduced AM isolate revealed a considerable tolerance to the pollutants. The response of AM colonisation to the pesticides varied with the poplar clones. Significant decrease of fungal and bacterial specific lipid fatty acids in the contaminated soils was also observed.

P0192. Interaction of endomycorrhizal fungi and salt stress on nutrient acquisition and growth of *Pistacia vera* L.

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Salt stress is considered as one of the most important abiotic factors limiting plant growth and yield in many areas of the world. It has been shown that vesicular arbuscular mycorrhizal (VAM) fungi decrease plant yields losses under salinity. The effects of VAM *Glomus etunicatum* inoculation on growth and mineral acquisition of *Pistacia vera* L. grown under salinity condition was studied. Different concentration of NaCl as 0(control), 50(low), 100 (medium) and 200mM(high) were employed for salinity stress. Plants were grown in a sterilized, low-P sandy soil in a greenhouse. The mycorrhizal colonization was higher in the control than in saline soil conditions. Dry weight of shoots, roots and also leaf area of mycorrhizal(M) plants were higher than nonmycorrhiza(NM) ones in both control and salinity conditions. The contents of N, P, K, Zn and Fe were higher in M than NM plants in control, low and medium salinity conditions. Concentration of Na in shoots of M plants was lower than NM grown under salinity condition. Generally, it can be said that M plants of *Pistacia vera* showed higher tolerance toward salinity than NM plants and their growth improved by VAM colonization.

P0193. Involvement of auxins in signaling during AM symbiosis

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The establishment of an arbuscular mycorrhizal (AM) symbiosis requires a continuous exchange of signals between host roots and fungi. Plant hormones might be suitable candidates for such signals. Inoculation of *Zea mays* with *Glomus intraradices* resulted

in the significant increase in the percentage of lateral roots during early stages of colonization which coincided with an increase in the levels of the auxin indole-3-butyric acid (IBA). Addition of TFIBA, an inhibitor of IBA-induced root growth and lateral root induction, to roots inoculated with AM-fungi reduced the formation of fine roots and the amount of endogenous free IBA as well as the percentage of colonization. We have started to isolate transcripts from maize and the model legume *Medicago truncatula* roots differentially induced by IBA and AM by cDNA-AFLP and microarray analysis. In addition, we have begun to analyze a family of five auxin conjugate hydrolases from *Medicago truncatula* biochemically and compared their expression during AM and nodule formation. Their differential expression during nodulation indicate that they might be important players to regulate auxin homeostasis in a symbiosis.

P0194. Mycorrhizal status of four species of a rare plant community in a Natura 2000 habitat

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Arbuscular mycorrhizal fungi (AMF) appear to influence plant community structure and diversity. Hence it is of special importance to analyse natural fungal communities in order to be able to provide inoculum if needed.

We determined the mycorrhizal status of *Laserpitium siler*, *Teucrium montanum*, *Acinos alpinus* and *Sesleria varia*, species of the rare plant community *Laserpitietum sileris* developed on an alpine chalk gravel slope. Root and soil samples were collected at three phenological stages of plant development and AMF spores were isolated. Colonization intensity by AMF and dark septate endophytes (DSE) was determined and roots of the same plant individuals were used for an amplification of partial sequences in the AMF rRNA genes.

Roots of all plants were colonized by AMF and DSE throughout the growing season with the highest values of total colonization on fructiferous plants. For an indication of the biodiversity of AMF present in roots molecular data will be presented and discussed.

P0195. The regulation of mycorrhization in supernodulating soybean mutants

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In the Rhizobium-legume interaction a so-called autoregulatory mechanism is well documented. Autoregulation of nodulation means, that already existing nodules systemically suppress subsequent nodule formation in other parts of the root system. Mutants of some legumes lost their ability to autoregulate the nodule number and thus display a supernodulating phenotype.

An autoregulatory mechanism has also been reported for the AM symbiosis. In a split-root system prior root colonization on one side suppresses further root colonization on the other side. Interestingly an established mycorrhizal association can systemically suppress not only further mycorrhization but also nodulation, pointing towards similar regulatory mechanisms in the two associations.

We studied the autoregulation of mycorrhization in supernodulating soybean mutants and soybean wildtype plants in order to see whether the rhizobial and the mycorrhizal autoregulatory mechanisms are linked.

P0196. Isolation of radish genes involved in phytochelatin synthesis and their possible use in phytoremediation via transplastomic technology

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Phytochelatin (PCs), i. e. (gGlu-Cys)₂-11-Gly, are a family of metal-complexing peptides, and play an important role in detoxification of toxic metals in plants. PCs are enzymatically synthesized from glutathione (GSH) or GSH-related compounds, and they form stable complex with heavy metals in the cytosol. These complex are subsequently sequestered into the vacuole. In this paper, we report the isolation of three cDNAs encoding enzymes, g-glutamyl-Cys synthase (g-ECS), glutathione synthetase (GS) and phytochelatin synthase (PCS), from radish, which involved in phytochelatin synthesis, and describe an attempt to integrate these genes into the chloroplast genome of tobacco. We also report an attempt to obtain a plant containing a synthetic oligonucleotides to produce a synthetic phytochelatin (EC8) in the

chloroplast. The purpose of present study is to obtain genetically engineered plants showing higher concentration of PCs in the plastid. The possible use of these plants for phytoremediation will be discussed.

P0197. Molecular and genetic diversity of a genomic sequence similar to SHATTERPROOF (SHP1) in Phaseolus vulgaris L.

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Compared with their wild progenitors, domesticated crops show marked phenotypic differences even if they belong to the same biological species. These differences, known as the domestication syndrome, results from selection for adaptation to cultivated environments. One of the key traits of the domestication syndrome is the seed dispersal mechanism (shattering), which was lost in common bean and in many other domesticated species. Two MADS-box genes involved in the control of silique shattering have been identified in *Arabidopsis thaliana* (*SHP1* and *SHP2*). Based on EST sequences, we identified in *Phaseolus vulgaris* two genomic sequences similar to *SHATTERPROOF (SHP1)* of *Arabidopsis thaliana*. We were also able to amplify and sequence a single product from several genotypes of *Phaseolus* spp. and a few other legumes. Furthermore, we developed two markers spanning indels that were used to locate the identified sequence on the *Phaseolus vulgaris* core linkage map (BAT93 X Jalo EEP558) and study its molecular diversity in a larger sample of wild and domesticated accessions from both Andean and Mesoamerican gene pools.

P0198. Transposable element domestication in plant genomes

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While transposons have traditionally been viewed as genomic parasites or junk DNA, the discovery of transposon-derived host genes have fuelled the ongoing debate over the evolutionary role of transposons. In particular, mobility-related open reading frames have been known to acquire host functions. We report that genome-wide searches for *Mutator* transposase-derived host genes in *Arabidopsis thaliana* (Columbia-0) and *Oryza sativa* ssp. *japonica* (cv. Nipponbare) (domesticated rice) identified 121 sequences, including the taxonomically conserved *MUSTANG1*. Syntenic *MUSTANG1* orthologues in such varied plant species as rice, poplar, *Arabidopsis*, and *Medicago truncatula* appear to be under purifying selection. However, *MUSTANG1* belongs to one of only two *Mutator*-like gene families with members in both monocotyledonous and dicotyledonous plants, indicating that *Mutator*-like elements seldom evolve into taxonomically widespread host genes.

P0199. How genes paint grape berry

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Anthocyanins are the main pigments in flowers and fruits. In grape the quantity and composition of anthocyanins influence berry skin colour. *VmybA* genes, belonging to C1 family, are involved in the regulation of anthocyanin biosynthesis in grape via expression of the UFGT gene. In particular a retrotransposon (Gret1)-induced mutation in *VmybA1* seems to be associated with the loss of pigmentation in some white cultivars of *Vitis vinifera*

We want to verify the presence of 3'LTR of Gret1 in some cultivars from North-Est Italy to investigate if black cultivars are evolved from white ones. Furthermore, in order to verify the "Gret1 hypothesis", an expression analysis of the genes belonging to the anthocyanin pathway is in progress in a progeny of a cross between a white and a black cultivar and in 30 different cultivars using Real Time-PCR. Our preliminary results indicate a direct correlation between a *MybA* gene and berry colour. Moreover the alignment of the 5' region of *VmybA* gene amplified from white and black cultivars induces us to hypothesize a different genetic event, besides the retrotransposon, between white and black cultivars in the coding sequence of *VmybA*

P0200. Gene duplication by rice Mutator-like DNA transposons: implications for host gene evolution and function

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Mutator-like elements (MULEs) containing duplicated host gene fragments have been documented in maize, *Arabidopsis* and rice, but the evolutionary impact of this phenomenon is presently unclear. In a genome-wide analysis of rice MULEs, we have identified 1,337 elements containing duplicated host gene fragments, 5% of which are expressed. Sequence analysis of the expressed duplications strongly suggests that they do not encode functional proteins; nevertheless, it does not exclude the possibility of a function at the RNA level. Endogenous non-coding transcripts are known to suppress the expression of genes with which they share short regions of high nucleotide sequence similarity, and increasing evidence indicates that this mechanism is much more pervasive than initially believed. We show that transcribed MULE duplications possess features that could allow them to regulate the expression of rice genes in a manner analogous to miRNAs and endogenous trans-acting siRNAs. Since their expression is regulated differently from the progenitor host genes, they may provide an additional level of complexity to gene regulatory networks.

P0201. Identification of Genes Relating to the Sexual Reproduction of a Unicellular Charophycean Alga, Closterium peracerosum-strigosum-littorale complex, by Microarray Analysis.

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Heterothallic strains of *Closterium peracerosum-strigosum-littorale* complex (*C. pslc*) are unicellular charophycean algae, having two sexes: mating type plus (*mt*⁺) and mating type minus (*mt*⁻). Two sex pheromones (PR-IP and PR-IP Inducer) involved in the progress of sexual reproduction of the *C. pslc* have been well characterized. The PR-IP inducer induces the release of PR-IP from *mt*⁺ cells, whereas PR-IP induces the release of gametic protoplasts from *mt*⁻ cells, during the sexual reproduction. To understand genetic program concerning the sexual reproduction, cDNA microarrays containing a total of 2806 cDNAs were prepared. Cy-5-labeled cDNA populations were prepared from cells proceeding sexual reproduction and cells treated with purified sex pheromones. As a result, 44 genes that showed a 4-fold increase in expression over the time-course were identified. Among them, expression levels of 11 genes were elevated only in *mt*⁻ cells by the addition of PR-IP, whereas that of 20 genes including a putative receptor-like protein kinase were elevated only in *mt*⁺ cells by the addition of PR-IP inducer. Further detailed analyses are now in progress.

P0202. Desiccation-Responsive Gene Expression In An Intertidal Brown Alga (Heterokontophyta): Population-Level Expression Patterns And Sequence Variation.

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Stress-tolerant brown algae in the genus *Fucus* are subject to recurrent desiccation during low tides in the intertidal zone (losing up to 95% of their tissue water). However, almost nothing is known about the molecular response to stressors in these algae. The species *F. vesiculosus* is widely distributed, from Arctic to warm temperate shores, and from marine to low salinity habitats (estuaries and the Baltic Sea). We constructed subtractive cDNA libraries from several populations responding to desiccation stress, and conducted within- and between-population screening of positive clones on macroarrays. The results suggest considerable variation in transcript profiles between populations from contrasting locations/habitats, when tested under common-garden conditions. While few genes could be identified directly from the SSH clones, analysis of an EST set of over 1000 sequences from 3 libraries

showed considerable variation within contigs (base substitutions, indels, and evidence for alternative transcription products), some of which were population-specific. Progress in gene annotation and the potential for marker development are reported.

P0203. Photosynthetic activity and primary vs. secondary pigments of the alpine snow alga *Chlamydomonas nivalis* cf.

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Snow algae inhabit most of the cold regions worldwide where long lasting snow fields are common. Ecophysiology of snow algae has been studied intensively in North America and occasionally at polar regions. Snow algae from European Alps have been studied mainly by means of light microscopy to describe systematic relationships.

Our studies on red snow algae (*Chlamydomonas nivalis*) from the high Alps of Austria show data on temperature and light dependence of photosynthesis as well as plastidal and extraplastidal pigment composition.

Both photosynthesis and respiration data support the cryophilic adaptation of snow algal cells, nevertheless *C. nivalis* produces oxygen without any inhibition up to 20°C for one hour or at irradiation up to 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Chlorophyll and primary carotenoid pigment composition is the same as in most *Chlorophyta*. Additionally, a high amount of free and esterified astaxanthin was found, located in cytoplasmic lipid globules. These pigments may shield the chloroplast against photoinhibition. Light microscopical observations show that the cell walls are frequently covered with tightly bound inorganic particles.

P0204. Compartmentation of the glycolytic pathway in heterokontophytes

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In heterokontophytes we characterized gene families encoding multiple (up to five) distinct isoforms of the glycolytic enzymes triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, enolase and pyruvate kinase. By employing GFP reporter gene techniques we were able to show that for each enzyme one isoform is transported into mitochondria of the diatom *Phaeodactylum tricornutum*. This strongly supports our previous suggestion (Liaud et al 2000) that at least the C3-part of glycolysis from triosephosphate to pyruvate runs through mitochondria in heterokont algae. Moreover, we were able to show by electron microscopy that envelopes from chloroplasts (4 membranes) and mitochondria (2 membranes) from *Phaeodactylum tricornutum* are closely attached. The stacked envelope membranes contain channels connecting both organelles suggesting a direct metabolic link between photosynthesis and respiration. These data suggest that the host cell of heterokont algae had mitochondria with a (partial) glycolytic pathway. Liaud et al.(2000); Mol.Biol.Evol. 17, 213-223.

P0205. Novel allergens of raspberry are cross-reactive with homologues in birch pollen and apple fruits

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Recently the description of occupational asthma due to the inhalation of raspberry powder was reported by Sherson et al., but no raspberry allergen has so far been identified and characterized. Our aim was to identify allergens of raspberry that can cause clinical symptoms and to investigate cross-reactivity of the raspberry allergens to birch pollen allergens.

Raspberry fruit extracts were prepared and subjected to SDS-PAGE. Immunoblot analysis with a sera pool of birch pollen and apple fruits allergic patients showed several IgE binding components with molecular weights ranging from 9 to 48 kDa. The most frequently detected protein had a molecular weight of 34 kDa and significant homology to the hevine family of latex allergens. Another detected protein with a molecular weight of 17 kDa had significant homology to the major latex-like protein and the Bet v 1

allergen family. Immunoblot with a mouse anti-Bet v 1 monoclonal antibody showed strong binding to the 17 kDa and 48 kDa proteins. We describe the initial identification of allergens in raspberry that may be important as cross-reactive allergens and novel members of the birch pollen-related food allergens.

P0206. Molecular aspects of plant food allergens

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Plant food allergens belong to a very limited number of protein families and many possess distinct biochemical and physicochemical properties. These include thermal stability and resistance to proteolysis which are enhanced by an ability to bind ligands such as metal ions, lipids, or steroids. Other types of lipid interaction including membranes or lipid vesicles may promote the allergenic properties of certain food proteins. Structural features related to stability are intra-molecular disulfide bonds and N-glycosylation. Some plant food allergens such as the cereal seed storage prolamins are rheomorphic proteins with polypeptide chains that adopt a variety of secondary structures resembling unfolded or partially folded proteins. Another type of plant food allergen is characterized by the presence of repetitive structures, the ability to form oligomers and the tendency to aggregate. A summary of the current knowledge regarding molecular properties of food allergens will be presented.

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P0207. Recombinant food allergens and hypoallergenic variants

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The physicochemical characterization of food allergens uses methods such as circular dichroism, mass spectrometry, 2D-gel electrophoresis, N-terminal sequencing and HPLC analysis. For some of the allergens experimentally based structures are already available. This information can be used to determine the relevant B- and T-cell epitopes leading to the prediction of hyper- and hypoallergenic members of a protein family. Five point mutations were introduced in the Mal d 1 wildtype, the major apple allergen and the resulting mutant was produced as a recombinant protein in *E.coli* and the allergenic properties of both proteins were investigated in IgE-immunoblotting assays, ELISA, peripheral blood monocytes proliferation assays and skin prick tests. Significant differences were found between Mal d 1wt and Mald 1mut. Our findings suggest that it is possible to modulate the IgE binding properties of allergens by single amino acid substitutions at crucial positions. Such mutated proteins may serve as a template for engineering hypoallergens for immunotherapy retaining T cell reactivity but decreasing the risk of anaphylactic side effects.

P0208. Localisation of the major allergen Art v 1 in *Artemisia vulgaris* and transgenic tobacco plants

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The major allergen of *Artemisia vulgaris* (mugwort) pollen, Art v 1, is a 108 amino acid protein consisting of two domains: 1. a cysteine-rich N-terminus with sequence homologies to members of the plant defensin family and 2. a highly glycosylated, proline-rich C-terminus domain containing several extensin-like repeats (Himly et al. 2003, FASEB J. 17: 106-108). Additionally, a signal peptide at the N-terminus was identified that directs the processed Art v 1 to the secretory pathway. Despite of the sequence homologies of the two domains, the physiological role of Art v 1 is still very unclear. To get more information on the possible function, the expression of the native Art v 1 protein was monitored in various tissues (root, stem, leaf, flower, pollen) of mugwort at the RNA and protein level. Using RT-PCR and immunodetection methods, Art v 1 was only detected in mugwort pollen. Additionally, recombinant

Art v 1 was produced in transgenic tobacco plants to obtain sufficient amounts of recombinant Art v 1 for testing its putative anti-fungal and anti-bacterial activity.

P0209. TPK/KCO family: K⁺ channels in *Arabidopsis thaliana*

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In *Arabidopsis thaliana* six members of structurally divergent potassium channels have been identified by sequence homology to AtTPK1, formerly described as AtKCO1. The five TPK-like proteins present four transmembrane segments and two K⁺-selective P-domains as their basic subunit structure. AtKCO3 presents only two transmembrane segments and a single K⁺-selective P-domain. Among the six channels, five show the presence of Ca²⁺-binding motifs in the C-termini and a putative 14-3-3 binding site in their N-termini.

Our project aims to analyse the function of the TPK/KCO channels using molecular, biochemical and physiological methods in transgenic plants including detailed expression analysis, subcellular localisation studies and reverse genetic approaches. Studies on AtTPK/KCO-GFP fusion proteins demonstrated that e.g. AtTPK1 is localised in the vacuolar membrane and AtTPK4 in the plasma membrane. Methods like Split-YFP-system, RET and THS are used to investigate protein interaction within the family and with regulatory proteins e.g. 14-3-3 proteins. Results from the molecular characterisation of this gene family will be presented.

P0210. Determination of Golgi targeting signals from a mannosidase II from *A. thaliana*

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Arabidopsis thaliana Golgi α -mannosidase II (GMII) is a glycosylhydrolase that is present in plants as well as in mammals and is involved in the formation of complex N-glycans, the major posttranslational modification of proteins. In order to examine Golgi targeting and retention domains of GMII, the putative cytoplasmic (C)-transmembrane (T)-stem (S)-region (CTS) of the enzyme and deletions thereof were fused to GFP and transiently expressed in the tobacco-related model species *Nicotiana benthamiana*. Confocal laser scanning microscopy analyses of the fusion proteins CTS-GFP, CT-GFP, TS-GFP, T-GFP, and C-GFP as well as the fusion constructs C10TS-GFP and C10T-GFP that included only 10 amino acids of the C region, revealed that the C10T region is sufficient to sustain Golgi retention of GMII. The T domain alone exhibited an incomplete retention, whereas the C region showed a homogenous staining of the cytoplasm suggesting that this region alone did not confer any Golgi retention by itself. These data indicate the definitive requirement of the T domain in Golgi targeting/retention and that the 10 amino acids of the C region confer an auxiliary role.

P0211. Ultrastructure of chromoplasts in *Crocus sativus* L., *C. cartwrightianus* Herb. and *C. thomasii* Ten.

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Crocus sativus contains crocetin and crocin carotenoids inside chromoplasts of red stigmas. Saffron is known only as cultivated species. Cytogenetical and molecular biology studies have led to the hypothesis that saffron originated by mutation or hybridisation of *C. cartwrightianus*. But also *C. thomasii*, which morphology is more similar to saffron, has been considered as ancestor. In this study the ultrastructure of chromoplasts of *C. sativus*, *C. cartwrightianus* and *C. thomasii* stigmas have been examined. Chromoplasts of *C. sativus* result in many vesicles and plastoglobules. Chromoplasts of *C. cartwrightianus* have plastoglobules, tubules and one or more dark body, spiral shaped, often crossing all the chromoplast. *C. thomasii* chromoplasts have mainly plastoglobules, electrontransparent vesicles encircled in a thin envelope, some thin fibrils and characteristic tubules heavy twisted. The comparison of chromoplasts of the supposed related species indicates that although similar is the basic organisation, each species has some different elements and, on whole, chromoplasts of *C. thomasii* are more similar to those of *C. sativus*.

P0212. Different Subcellular Localisation of Two Arabidopsis PP2C Phosphatases

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Studies on plant protein phosphatases type 2C (PP2Cs) suggest that they are involved in the regulation of diverse signaling pathways. *Arabidopsis* contains the largest PP2C family in Eukaryota. However, the information about them is very limited. AP2C1 specifically interacts with and negatively regulates the activity of stress-activated MAPKs. Function of AP2C3, a homologue from the same cluster, is not known yet. One of the key characteristics of proteins, which can help to elucidate their possible functions, is their subcellular location. Here we analyse intracellular localisation of these two protein phosphatases in plants by protein fusions with GFP. AP2C3-GFP protein is localised to the nucleus suggesting that it may act on MAPKs within this compartment and can possibly be involved in the regulation of gene expression. AP2C1-GFP tagged proteins could be detected in a highly dynamic cellular compartments that most resembles newly re-discovered structures, called stromule. It is thought that stromule production may be essential for optimising signal transduction processes that require intracellular movement of substrates or signals.

P0213. Reconstruction of spatial structure of plant FtsZ-proteins

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3-D models of two forms of plant FtsZ-proteins localized in chloroplasts and in cytosol of *Arabidopsis thaliana* L. were developed. Crystal structure of bacterial FtsZ-GDP complex (PDB-code 1FSZ) was used as matrix. Secondary structures of both plant protein forms contain ten α -strands. However chloroplast molecule forms ten α -helices and four 3_{10} -helices, whereas in cytosolic molecule one α -helix (0-helix before GDP-binding domain) and C-terminal 3_{10} -helix are absent. It was found that T2-loop structure of nucleotide-binding pocket of chloroplast FtsZ-protein contains non-charged amino acid residue (Ala) in position 111 instead charged amino acid residue in cytosolic form (Arg) and polar residue in bacterial FtsZ-protein (Gln) in respective positions. Accounting relatively low sequence homology of plant and bacterial FtsZ-proteins (~47%) developed 3-D models demonstrate high coincidence not only between structures of whole molecules FtsZ-proteins and bacterial matrix, but between structures of nucleotide-binding pockets as well. Respectively, they can be used for further modeling of plant FtsZ-proteins with specific low molecular ligands.

P0214. Identification of plastid envelope proteins required for the import of protochlorophyllide oxidoreductase PORA into the chloroplast of barley and *Arabidopsis*

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Chloroplasts are semiautonomous cell organelles which must import the major part of their protein constituents from the cytosol. Import is for most cytosolic precursors dependent on ATP and mediated by cleavable amino-terminal transit sequences. Biochemical and genetic evidence in *Arabidopsis thaliana* suggests the existence of multiple protein import complexes which could differ by an interchange of receptor components. Here we report that the NADPH:protochlorophyllide (Pchl) oxidoreductase precursor, pPORA, uses a distinctive import site. Chemical crosslinking and various other approaches revealed that pPORA interacts with several hitherto unknown components. Among them is a 47 kDa protein implicated in synthesis of photoprotective vitamin E and a 52 kDa Rieske type non-heme iron oxygenase related to the inner plastid envelope translocon protein Tic55, the lethal leaf spot protein Lls1 and chlorophyllide a oxygenase (Cao), all of which have been localized to chloroplasts. It is the aim to discuss the role of Ptc52 in the regulation of pPORA import.

P0215. Analysis of a putative plastidic transporters affecting photosynthesis in *Arabidopsis thaliana*

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Membranes are the major barriers between the cytoplasm and cell organelles. Therefore, a variety of transporters are needed to mediate the exchange of metabolites, ions and proteins between these compartments.

We are analysing a family of five proteins with putative transport function in *Arabidopsis thaliana*. A comparison between several prediction programmes (<http://aramemnon.botanik.uni-koeln.de/>) revealed that these proteins contain more than five transmembrane spanning regions and different subcellular targeting signals. For each member homozygous T-DNA insertion mutants were isolated. Plastidic targeting for two proteins of this family (AtDAP1, AtDAP2) was confirmed experimentally by GFP fusion constructs. The corresponding T-DNA insertion mutants exhibit drastic phenotypes characterised by dwarfish growth, reduced chlorophyll and starch content and a significantly lowered electron transport rate. Therefore, these mutants are named *dwarf* affected in photosynthetic electron transport rate (DAP).

P0216. The plastidic phosphoenolpyruvate/phosphate translocator (PPT) in *Arabidopsis*: a regulatory element of mesophyll development?

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A. thaliana contains two PPT genes. *AtPPT1* is mainly expressed in the vasculature of leaves and roots, but not in mesophyll cells whereas *AtPPT2* is expressed throughout the leaf blade, but not in roots. The *A. thaliana cue1* mutant, lacking PPT1, exhibits a reticulate leaf phenotype with dark green paraveinal and pale green interveinal regions. This phenotype could be rescued transiently by feeding aromatic amino acids or by overexpressing plastid-targeted pyruvate, orthophosphate dikinase producing PEP from pyruvate in the stroma. This observation reinforced the proposed role of PPT as the provider of PEP to plastids as precursor for the shikimate pathway. However, contents of aromatic amino acids and secondary plant products were only selectively affected in *cue1*. We propose that the absence of *AtPPT1* leads to a deficiency only in certain products of the shikimate pathway, the synthesis of which is restricted to certain cell types. It is likely that derivatives of the phenylpropanoid metabolism fulfill hormonal or signal-like functions triggering correct mesophyll development. The impact of a candidate compound on the transcriptome will be presented.

P0217. Isolation and characterization of *Arabidopsis* apm mutants with defects in peroxisome biogenesis

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Plant peroxisomes have a variety of functions in various metabolic pathways, including metabolism of storage oils, photorespiration, scavenging hydrogen peroxide and biosynthesis of jasmonic acid. Based on the observation of peroxisomes visualized with GFP in *Arabidopsis*, it is revealed that peroxisomes change the size, shape, number and position within the cell. Little is, however, known about the mechanism for peroxisome biogenesis in higher plants. To identify the genes controlling peroxisome biogenesis, we isolated a number of *Arabidopsis* mutants with aberrant peroxisome morphology (apm mutants). apm mutants were categorized into four classes as follows, (1) long peroxisomes (apm1); (2) larger peroxisomes (apm3, apm6); (3) altered distribution in cells (apm5); and (4) observation of GFP fluorescence in the cytosol as well as in peroxisomes (apm2, apm4, apm7). Among these mutants, we reported that apm1, whose peroxisomes are long and reduced in number, had the mutation in dynamin-related protein 3A (DRP3A), one of sixteen dynamins in *Arabidopsis*. In this presentation, we would like to discuss the phenotype of apm mutants.

P0218. Analysis of new protein families homologous to plastidic phosphate translocators in *Arabidopsis thaliana*

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Several proteins (PTh) that show homology to nucleotide-sugar transporters (NSTs) and to plastidic phosphate translocators (pPTs) were recently identified in *Arabidopsis* and other organisms. The PTh proteins split into three new families named the KD, KT and KV/A/G families according to conserved sequence motifs that might be involved in substrate binding. Two of these transporters belonging to the KV/A/G family possess a N-terminal presequence that might direct the proteins to plastids. In order to analyze the function of these two proteins, the cellular and tissue specific expression of the corresponding genes were determined by RT-PCR and promoter-reporter gene fusion studies. Furthermore, transport experiments with the heterologously expressed proteins should shed light on the substrate specificities of these transporters.

The characterization of mutant lines with insertions in these genes revealed no distinct phenotype, suggesting overlapping functions of the proteins. The analysis of mutants lacking more than one gene might provide new insights in the physiological role of these newly identified transporters.

P0219. Functional Characterization of *Arabidopsis* Proteins homologous to Bile Acid Transporters

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The Na⁺-Bile Acid Transporter (BAT) family contains over 50 members in animal, plant and bacterial species. BATs in mammals catalyze an electrogenic Na⁺-bile acid symport in intestinal, liver and kidney tissues. These transporters exhibit broad specificity, taking up taurocholate and other bile acids as well as a variety of other organic compounds. *A. thaliana* possesses a small BAT family consisting of five proteins. All of them have N-terminal presequences directing the proteins (and marker proteins like GFP) to plastids and mitochondria. Obviously, in plants, in contrast to animals, these proteins are exclusively located in cellular organelles. To elucidate the physiological function of these transporters, different knock out lines of the candidate genes were analyzed to complete the picture we got from expression studies. Furthermore, transport experiments with the heterologously expressed proteins should shed light on the substrate specificities of these transporters.

P0220. Peroxisomal citrate synthase and peroxisomal malate dehydrogenase are required for fatty acid metabolism in *Arabidopsis*

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Arabidopsis seeds in which two peroxisomal citrate synthase (*pCSY*) genes are knocked out are dormant, and they do not metabolise their stored lipid. Germination can be achieved by removing the seed coat and supplying sucrose. The mutant seedlings are resistant to 2,4-dichlorophenoxybutyric acid (2,4-DB), indicating a block in peroxisomal α -oxidation. Therefore *pCSY* is not just a glyoxylate cycle enzyme but is also required for fatty acid respiration and to break dormancy. It is hypothesised that peroxisomal malate dehydrogenase (*pMDH*) serves to oxidise NADH produced by α -oxidation, and does not oxidise malate to provide oxaloacetate for the glyoxylate cycle. *Arabidopsis* mutants in which two *pMDH* genes are knocked out are unable to establish as seedlings unless exogenous sucrose is supplied. Seedlings are insensitive to 2,4-DB, showing that α -oxidation is defective, and fail to metabolise stored lipid. The metabolism of [2-¹⁴C]-acetate into sugars and organic acids is normal in mutant seedlings, indicating that the glyoxylate cycle is active. These results show that *pMDH* is not required for the glyoxylate cycle, but is required for fatty acid α -oxidation.

P0221. Characterization of the plant ESCRT-III complex: protein interaction studies between its putative Arabidopsis thaliana components

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Protein sorting to multivesicular bodies (MVBs) requires the coordinated action of three protein complexes, ESCRT-I, -II, -III (Endosomal Sorting Complex Required for Transport). Based on analyses in yeast, up to 14 class E VPS (Vacuolar Protein Sorting) proteins have been assigned to this protein degradation and recycling pathway. A current model proposes that sorting starts with the binding of VPS27 to ubiquitinated proteins which recruits ESCRT-I to MVB membranes and activates ESCRT-II. Later it initiates the assembly of at least four small coiled-coil proteins forming ESCRT-III which separates after VPS4 association. In plants nothing is known about the presence and function of an ESCRT mediated MVB protein sorting pathway. In comparative genome analyses we identified all putative homologs of the ESCRT proteins in the fully sequenced genomes of Arabidopsis and rice. To further gain insights into the composition of ESCRT-III we carried out more than 100 yeast two hybrid protein interaction studies with its putative Arabidopsis components and propose a model for their arrangement in a plant ESCRT-III complex. Supported by the Austrian Science Fund (FWF).

P0222. Induction of dehydrin-like protein in rice seedlings under low temperature

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Proteomic analysis was conducted on rice seedling grown under abiotic stress in relation to dehydrin induction. Dehydrin, a group 2 late embryogenesis abundant protein, is supposed to have protective functions in plant tissues. After germination under salt-containing medium, rice seedlings were transferred to low temperature. Proteins were profiled by two-dimensional electrophoresis and immunoblotting with antibody for conserved lysine motif of dehydrin. Growth of the seedlings was remarkably inhibited with 0.8% NaCl. The electrophoresis profile of seedlings on 0.8% NaCl showed weaker spots for 16, 18, 27 kDa polypeptides and denser spots for 61kDa and 36-37kDa proteins than control. No significant difference in protein profile was observed between the seedlings grown on 0 and 0.5% NaCl. After they were incubated at low-temperature, however, increase of heat-stable proteins and induction of acidic dehydrin were observed in those transferred from 0.5% NaCl medium. The induction of dehydrin in acclimated seedlings implied the possibility to enhance the low temperature tolerance of rice seedling by moderate salt treatment.

P0223. Provision of anti p5cs and application of it in the analysis of the expression of the p5cs gene in transgenic versus nontransgenic olive under osmotic stress.

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Osmotic stress is an important limiting factor. To cope with these environmental stresses, application of molecular plant breeding is necessary. In response to osmotic stress elicited by condition of high salt, the expression of P5CS gene altered in olive plants. For this, constructs (S,E,X) were prepared by introducing P5CS sequence in to PBI121. Constructs cloned in binary vector were transformed to the disarmed agrobacterium tumefaciens. Olive embryo were transformed with A.tumefaciens harboring the various gene constructs and salt-tolerance plants were regenerated in media containing adequate concentration of salt. The total protein was extracted from fresh leaves of both control and transformed regenerated plants. and measured using of bradford to subjected to two dimensional polyacrylamide gel electrophoresis.

To probe the effect of p5cs overexpression on salinity stress tolerance, poly clonal antibody developed in rabbit against p5cs protein. The result of immunological methods for specific detection of p5cs gene, strongly indicated P5CS up- regulation in stressed transformed plants versus non transformed plants.

P0224. Differential polypeptide expression in olive plantlet under osmotic stress

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For study olive plantlet response under osmotic stress condition. In first step the embryos of the olive C.V.Zard were cultured on solid 1/2MS media, after one month, some of the green plantlets were transferred to the solid 1/2 MS containing 200 mM NaCl, the plantlets were subcultured weakly, after two weeks, according to different components of olive plantlets, several methods used to extract total proteins from both plantlets under stress condition and non stress condition. Equal quantity of total protein from both two samples was subjected to 2DE and protein profile of stressed plantlets was compared to non-stressed plantlets. Appearance, disappearance, up and down regulation of polypeptides were interpreted as response of plantlet to the induced stress and the difference in polypeptides profile of plantlet under stress versus control plantlet was also considered. Along with the above observation a significant difference in specific polypeptides pattern of stressed plants was observed with that of control indicates that effect of salinity on gene expression in olive plantlets.

P0225. Molecular mapping of genes related to salt tolerance in rice

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The present study was conducted to identify the genetic factors controlling the traits related to salinity tolerance. A total of 6 quantitative traits, including Sodium and Potassium concentration in root (NaR, KR) and shoot (NaS, KS), Na-K ratio in root and shoot, were scored in an BC₂F₄ population from a cross between IR64, Tarom Molaei. A total of 62 BC₂F₄ lines were genotyped by means of 114 SSR markers. Transgressive segregation was observed in all traits. Using Multiple Interval Mapping, a total of 16 putative QTLs were detected, of which 3 were for KS, 3 for NaS, 3 for KR, 4 for NaR, 1 for NaS/KS, 2 for NaR/KR. The amount of variation explained by individual QTLs ranged from a low of 9% to a high of 57%, and many of the QTLs accounted for more than 15% of the variation. This study indicated that favorable QTL alleles could be transferred from local variety into an improved variety for improvement of quantitative trait loci like salinity tolerance by the advanced backcross QTL strategy and molecular breeding.

P0226. Characterization and functional analysis of Arabidopsis thaliana MAP kinase MPK10

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Mitogen-activated protein kinase (MAPK) cascades are universal signal transduction modules present in all eukaryotes. These protein phosphorylation cascades integrate extracellular stimuli into cellular responses. In plants MAPKs are involved in the responses to various biotic and abiotic stresses, hormones, cell division and developmental processes.

The focus of our work is the *Arabidopsis thaliana* gene MPK10, a representative member of the group A MAPKs. We isolated and characterized knock-out mutant lines (SALK collection), via PCR and sequencing approaches. These analyses showed that expression of MPK10 is not impaired, however the insertion of the T-DNA into the gene leads to a shift of the open reading frame, thus to a non functional transcript. We also created over expression lines and dominant negative mutant versions with an amino acid exchanged in the catalytic site of the kinase. We isolated some transgenic lines, which we are currently characterizing.

P0227. Mechanisms, specificities and rates of nuclear DNA content variation in flowering plants

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We have used comparative analyses of orthologous genomic segments from several grass species to identify the rates and natures of indel generation. These results have been compared to changes in DNA sequence that we have observed in rice and *Arabidopsis* LTR-retrotransposons, a family of transposable elements that can serve as surrogates for the entire genome. The data indicate that genome growth is primarily associated with polyploidy and transposable element amplification. This growth competes with a slow but continuous loss of DNA sequence by small deletions, most associated with illegitimate recombination or unequal homologous recombination. Different species vary in their rates and primary mechanisms of both DNA addition and removal. Moreover, different regions and components within grass genomes undergo very different rates and dominant mechanisms of indel accumulation. These studies provide a mechanistic framework to understand the exceptionally dynamic genomes found in the nuclei of flowering plants.

P0228. 5S rRNA gene diversity in the genus *Secale* and which are its closest haplomes

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Secale contains the cultivated cereal Rye along with a number of wild species. This genus is a member of the Triticeae tribe. The purpose of the present investigation was to assess the diversity of the 5S DNA units within the genus, to determine haplomes closest to it also based on the 5S DNA units and to compare the findings with different taxonomies and position of the genus within the Triticeae tribe. *Secale* contains long R1 and short R1 units which although vary are not useful for distinguishing species and below. Phylogenetic inferences were drawn from Bayesian and maximum likelihood analyses. The closest related haplomes are the **P** haplome inferred from the 5S short R1 units, and the **St** haplome inferred from the long R1 units. Thus *Secale* appear to be phylogenetically close to the predominantly diploid genera *Pseudoroegneria*, containing the **St** haplome, and to *Agropyron* containing the **P** haplome. Somewhat less related are the **J** haplome, typical to *Thinopyrum*, and possibly the **V** haplome typical to *Dasyphyrum*. Traditional taxonomies classify *Secale* and *Dasyphyrum* (Mosquitograss) next to each other which is in some contrast with the present findings of the 5S DNA units.

P0229. Preliminary study of relative DNA content variation in some European populations of *Lactuca serriola*

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L. serriola is extremely variable wild species and is considered as a progenitor of cultivated lettuce (*L. sativa*). The investigation includes the analysis of relative nuclear DNA contents of 99 *L. serriola* accessions collected in 12 European countries. Results obtained by flow cytometry were based on DAPI staining technique. Data on 2C relative DNA contents showed significant variation (from 4.34 to 6.85) among individual populations. The set studied was divided into 21 groups differing in relative DNA contents. Significant differences in the mean DNA content were found among populations originating from different countries. One of the lowest values (4.66) is characteristic of material from Switzerland, the highest (6.85) for Slovak Republic. Nuclear DNA contents in *L. serriola* was not clearly related to their taxonomic status (*f. serriola*, *f. integrifolia*). Research was supported by projects MSMT 153100010 and MSM 6198959215.

P0230. Accumulation of chloroplast DNA sequences on the Y chromosome of the dioecious *Silene latifolia*

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Horizontal transfer of plastid and mitochondrial DNA sequences into plant nucleus is a well-described phenomenon. It has been accepted that the position of integrated organelle DNA is random and frequently directed to (peri)centromeric regions of all of the

chromosomes. The model dioecious plant *Silene latifolia* possesses a pair of heteromorphic sex chromosomes, X and Y, in an early stage of evolutionary divergence. The Y chromosome is largest in the *S. latifolia* genome and this fact could predict an accumulation of repetitive sequences in the Y-non-recombining region. In this study, we present a chloroplast derived BAC clone preferentially and strongly hybridizing to the Y chromosome of *S. latifolia*. Using X and Y laser microdissection followed by DOP-PCR we prepared DOP-X and DOP-Y libraries, which were later screened for chloroplast DNA sequences. By virtue of low sequence divergence of selected DOP clones and original chloroplast sequence, we come to a conclusion of a recent transfer of chloroplast DNA to the Y chromosome of *S. latifolia*.

P0231. The albino enigma - DNA deletions and translation-deficiencies in microspore-derived plants

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In monocotyledonous plants, which were regenerated from cultured microspores, albinism is a frequently observed phenomenon. Frequently, large rearrangements or deletions were found in the plastid genomes of microspore-derived albino plants. In a significant number of albino plants, however, no deletions could be detected. We examined gene expression in plastids of wheat albino plants. Analysis of steady-state RNA levels of several plastid genes showed a deviant transcript pattern compared to green plants. The altered plastid RNA profile can be explained by the lack of an active plastid-encoded RNA polymerase. Examination on the protein level revealed the absence of plastid-encoded proteins. Particularly, the absence of an essential component of plastid ribosomes strongly suggests a complete deficiency of plastid protein synthesis, which can explain the absence of the plastid-encoded RNA polymerase. These results indicate that monocots are able to survive without plastid protein synthesis when sugar is supplied in the growth medium.

P0232. Androgenesis in vitro of Siberian larch (*Larix sibirica* Ledeb.)

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At present androgenesis in vitro takes an important place in plant biology. It was induced in many angiospermous plants. With the help of androgenesis in vitro the selection process is accelerated, new sorts and lines of grasses are created. The male buds of Siberian larch do not have an organic dormancy in the fall-winter period and under favorable conditions they are able to complete the development of the male generative structures. Cultivation of microsporophylls on the MS medium containing 0,2-0,5 mg/l of hormone 2,4-D during a month results in induction of androgenesis in vitro in Siberian larch. The increase of hormone concentration causes development of necrotic processes. Data on androgenesis in vitro of Siberian larch, as of a representative of gymnosperms were obtained for the first time. At cultivating microsporophylls of Siberian larch on the MS medium with concentration 0,2-0,5 mg/l 2,4-D the embryos of two development types were obtained.

P0233. The ARIADNE family in *Arabidopsis thaliana*: A characterization of putative E3 ligases

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ARIADNE (ARI) proteins were recently identified in fruitfly, mouse and man. They are characterized by two C₃HC₄ RING-finger motifs separated by a linker region (IBR). ARI proteins share their RING-IBR-RING domain with PARKIN and there is increasing evidence that ARI/PARKIN proteins function as E3 ubiquitin ligases. On the basis of homology and motif searches, we identified 16 *AtARI* genes in *Arabidopsis*. Using real-time quantitative PCR we determine the expression level of the family in different organs. The subcellular localization of ARI1, ARI2 and ARI12 was studied with peptide-specific antibodies on *Arabidopsis* cell cultures and revealed that ARI1 has a speckled localization in the cytoplasm. ARI2 is found exclusively in the nucleus, whereas ARI12 exhibits nuclear and a weak speckled staining in the cytoplasm.

To address the function of this gene family we initiated a reverse genetic approach by screening for knock out alleles. The phenotypic characterization of single and the progress on double and triple mutant analyses will be presented.

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P0234. Two *A. thaliana* members of a novel protein family are potentially involved in nucleobases transport

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Nucleobases and derivatives play essential roles in the cells of all living organisms. In plants, crucial transport processes like the uptake of purines and pyrimidines for salvaging, the long distance transport of purine degradation products, and the allocation of cytokinins are not well understood. In this work, two novel putative transporters for nucleobases encoded in the Arabidopsis genome have been identified. Both coding sequences restored the capacity to take up adenine when expressed in a *S. cerevisiae* mutant defective in adenine uptake (*fcy2*). Competition studies showed that other purines and derivatives are also transported. To assess the subcellular localization, transient expressions in *N. tabacum* BY-2 protoplasts of GFP fusions under the control of the CaMV 35S promoter were conducted. RT-PCR analysis and promoter activity studies revealed the organ and cellular expression of both genes. Whereas one of the genes is expressed in all plant organs, the second shows a more regulated expression pattern, which is maximal in roots and flowers. Interestingly, expression of one member is strongly induced by exogenously applied auxin.

P0235. Comparison of detected QTLs for the traits pre-harvest sprouting and dormancy in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.)

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A set of 114 recombinant inbred lines (RILs) of the ITMI mapping population (International Triticeae Mapping Initiative, Mexico) was grown under field conditions in Gatersleben in 2003 and under greenhouse conditions in 2004. The lines were evaluated for the domestication traits pre-harvest sprouting and dormancy (germinability). Main QTLs could be localized for pre-harvest sprouting on chromosome 4AL and dormancy on chromosome 3AL. The location of the pre-harvest sprouting gene on chromosome 4AL found in other cultivars was confirmed. Also known dormancy effects on chromosome 3AL in the cultivar "Chinese Spring" were approved. In addition, 94 double haploid lines (DH) of the OWB-population (Oregon Wolfe Barley) grown under field and greenhouse conditions are in study to discover QTLs for these traits. The OWB-population is a well characterised, phenotypically polymorphic mapping population that has been used for a whole range of mapping and QTL studies. A comparison of the detected QTLs between wheat and barley is proposed.

P0236. Repetitive DNA behaviour induced by metals in *L. riparium* and *F. hygrometrica* gametophytes

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In order to investigate the relationship between environment and genome, we studied the repetitive DNA behaviour in answer to metals in two Bryophytes: the aquatic *L. riparium* and the terricolous *F. hygrometrica*. Working in *in vitro* cultured gametophytes, we showed that a direct "cause and effect" relation exists between metal stress and repetitive DNA behaviour. In fact, A-T and G-C specific fluorochrome treatment showed that cadmium or lead are able to induce a selective amplification of some GC-rich repetitive DNA sequences forming peculiar agglomerates inside the nucleus. As soon as the metals are removed from the culture medium, the DNA amplification stops and the new formed repetitive DNA, that is quantitatively proportional to the time of plant exposure to metals, is gradually eliminated from the cell. *In situ* hybridization and Southern Blotting experiments showed that also rDNA is involved in the described

phenomena. Electron microscopy and X-ray TEM microanalysis show that metals are able to reach heterochromatic areas of genome. Our data support the theories considering repetitive DNA as a possible mediator between environment and gene expression.

P0237. Identification of heat shock proteins in camu-camu (*Myrciaria dubia* (H.B.K.) McVaugh)

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The functional genome of camu-camu, native specie of the Amazonian, enables the identification of new genes of biotechnological interest by means of the sequencing of Expressed Sequence Tags (EST). The sequences were identified by the system of alignment tBLASTx - NCBI, and analyzed as regards the homology in the LALING program. The camu-camu EST project this in course, were present partial results of the identification of the ESTs that encoding heat shock protein. The *Md3i95* sequence was similar to sHSP17,5Cl of *C. papaya* (GI:38639430). The *Md3i403* clone was similar to sHSP17,6Cl of *L. esculentum* (GI:4836472). The *Md3i20* and *Md3i717* sequences were similar to sHSP18,5Cl of *G. max* (GI: 18653); and the *Md3i700* sequence was similar sHSP22 of *G. max* (GI:18660). The *Md3i732* sequence was similar to HSP70 of *V. labrusca* (GI: 14581676); and the *Md3i87* and *Md3i768* sequences were similar to HSC70-2 of *A. thaliana* (GI: 30679645) and HSC70-3 of *V. radiata* (GI: 45331284), respectively. The *Md3i152*, *Md3i09* and *Md3i85* sequences were similar, respectively, to HSP80 of *E. esula* (GI: 6934297), HSP82 of *N. tabacum* (GI:19879), and HSP83 of *P. nil* (GI: 169295).

P0238. QTLs contributing to pearl millet [*Pennisetum glaucum* (L.) R. Br.] grain yield under varying moisture conditions during grain filling

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The objective of this study was to identify QTLs for yield and yield-correlated traits that would maximize/stabilize grain yield across a range of moisture conditions during grain filling. The study used testcrosses of 78 skeleton-mapped F2-derived F4 progenies from the (ICMB 841-P2 × 863B-P1)-derived pearl millet mapping population. These were evaluated in managed fully-irrigated non-stress conditions, as well as late-onset (mild) and early-onset (severe) terminal drought stress conditions, over four years in the dry-season drought nursery at ICRISAT-Patancheru to map genomic regions associated with grain yield and related traits. Three QTLs for grain yield detected on LG1, LG2 and LG3 were stable across moisture environments and years and co-mapped with other yield-related trait QTLs. There was useful complementarity between the 863B alleles at a strong panicle harvest index QTL on LG1 and the harvest index QTL on LG2, and the ICMB 841 allele at the harvest index QTL on LG3. Stability of pearl millet grain yield under a range of grain-filling moisture conditions should be enhanced by marker-assisted pyramiding of the favorable alleles at these three QTLs

P0239. Isolation of putative type 2-metallothionein-encoding sequences and their spatial expression pattern in the seagrass *Posidonia oceanica* after metal treatment

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Metallothioneins (MTs) are proteins with low molecular mass and high cysteine contents that bind to heavy metals and probably play a role in their metabolism and detoxification. We isolated eleven MTs-like sequences from copper- or cadmium-exposed plants of the seagrass *P. oceanica*, a marine Angiosperm playing a major role in maintaining infralittoral ecosystems in the Mediterranean Sea. These sequences show high similarities with genes encoding type 2-MTs. Neighbor-joining analysis indicates that at least two subfamilies occur within *Posidonia* type 2 MTs, showing however a strong sequence uniformity. For each subfamily we analyzed two

gene (*Pomt2b* and *Pomt2f*) by Southern blotting and hybridization using a DIG-labeled riboprobe. The same riboprobes were used to determine, by *in situ* technique, the expression domain in *Posidonia* plants both *in vivo* and exposed to cadmium in aquarium. *Pomt2b* and *Pomt2f* show tissue specific expression and a high induction during Cd treatment. Our results suggest that *Pomt2b* and *Pomt2f* play an important role in maintaining the metal homeostasis and detoxification in *P. oceanica* shoot and leaves.

P0240. Characterization and tissue expression pattern of two aquaporin-encoding genes in the seagrass *Posidonia oceanica*

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Beyond simple diffusion across a lipid bilayer, water transport through cell membranes is related to aquaporins. Aquaporins belong to a highly conserved group of membrane Major Intrinsic Proteins. In contrast to terrestrial plants, no aquaporins or aquaporin-encoding genes have been isolated from marine plants. *Posidonia oceanica* is a marine angiosperm, largely widespread in the Mediterranean Sea. We have isolated by PCR-RACE two genes encoding putative proteinaceous water channels, *PoPIP1;1* and *PoTIP1;1*. *PoPIP1;1* is unique in the genome of *P. oceanica*, while *PoTIP1;1* belongs to a subfamily of at least four members. Both genes are constitutively expressed in the leaves, and are more transcribed in young than in differentiated tissues.

We have also determined, by *in situ* technique, the expression domain of *PoPIP1;1* e *PoTIP1;1* in different organs of the plant. *PoPIP1;1* is preferentially localized in the apical meristem and in the rhizome, whereas *PoTIP1;1* is localized in the root; both the aquaporins are expressed in the leaf mesophyll, suggesting an important role of these aquaporins in the uptake and water homeostasis in *P. oceanica* plant.

P0241. Distinct patterns of DNA methylation characterise populations of *Posidonia oceanica* (L.) Delile derived from anthropically stressed and intact areas.

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Posidonia oceanica (L.) Delile is an important marine phanerophyte of the Mediterranean area. In the last years a regression of meadows has been caused by the increasing anthropization and *Posidonia* has been proposed as an effective bio-indicator to monitor sea environments. DNA methylation is a fundamental mechanism for gene expression regulation and plant development. In this context, we investigated the changes in DNA methylation between two populations of *Posidonia* derived from anthropic-stressed and intact areas respectively during three different periods in the year.

DNA methylation of shoot apical meristems and young leaves was monitored by immunocytological and MSAP analysis. A set of morphometric and nucleotypic features were also investigated. The populations of anthropized meadows showed cytosine hypermethylation and chromatin re-modelling with respect to those of intact areas. Moreover, MSAP signal-band profiles revealed that the DNA methylation grade of the former was higher than controls, thus suggesting that the methylation increase in *Posidonia* may be used as a molecular marker to predict and evaluate altered environmental condition.

P0242. Assembly of a cDNA-microarray to test abiotic stress in pepper (*Capsicum annuum* L.)

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The aim of our study is the assembly of a stress-array with homologous and heterologous cDNA probes. Literature research resulted in more than 1000 interesting drought stress related genes in various species. Corresponding ESTs available in the PICME-clone depository (www.picme.at) were selected by BLAST alignment. Clones of differentially expressed genes of pepper isolated by cDNA-SSH serve as control. The stress-array will be evaluated with a stress-tolerant (Ziegenhorn Bello) and a stress-sensitive (Milder Spiral) pepper variety. To correlate gene expressions with the physiological status of the plants, selected

stress genes (real time-PCR) and proline content, peroxidase activity and osmotic potential were measured. In addition the effect of several endophytic bacteria on these two pepper varieties were tested. Preliminary tests revealed two strains of growth promoting bacteria, which will be further investigated.

P0243. Large scale screening for carotenoid genes in tomato: Map-based cloning and characterization of *white-flower* (*wf*) and *high-pigment 3* (*hp3*)

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To identify genes that regulate carotenoid biosynthesis in plants we have isolated novel mutations that affect colors of flower and fruit in tomato (*S. lycopersicum*, CV M82) by screening more than 200,000 tomato plants following EMS or fast-neutron mutagenesis. Twenty one mutants with altered flower pigmentation and 39 mutants with fruit color changes have been identified. Carotenoid composition in leaves, petals, anthers and fruits of each mutant was determined by HPLC. Two mutants, *high pigment 3* (*hp3*) and *white flower* (*wf*) were further studied. Mutant *hp3* accumulates 40% more carotenoids in the fruit compared with the wild type and its flowers and leaves contain high level of zeaxanthin and antheraxanthin at the expense of violaxanthin and neoxanthin due to a mutation in zeaxanthin epoxidase. Consequently, ABA is reduced in this mutant and its leaves are more sensitive to strong light. Wild-type tomato flowers accumulate high concentration of neoxanthin. In contrast, *wf* flowers contain low levels of -carotene. Cloning of *wf* revealed that it encodes a flower-specific -carotene hydroxylase. Tomato has two beta-carotene hydroxylase genes: one is constitutively expressed in leaves and the other is exclusively transcribed in flowers. The elucidation of the molecular basis of the *wf* mutation underscores the evolution by gene duplication of a chromoplast-specific carotenoid biosynthesis pathway.

P0244. Plant myosin VIII: from cell-cell communication to stress response

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AtATM1 is the first cloned and sequenced plant myosin and represents the founding member of the class VIII of myosins. Polyclonal antibodies have been raised against its unique C-terminal tail domain and used for the localization of AtATM1 via light and electron microscopy. AtATM1 localizes preferentially to the plasma membrane at cell periphery domains enriched with callose. In particular to the newly formed cytokinetic cell walls, auxin transporting end-poles in the root apex, phloem sieve plates, as well as to plasmodesmata and pit-fields of the inner cortex near unloading phloem elements of root apices. Moreover, it emerges as endocytic motor of plant cells. Under stress situations, like microgravity environment and hyperosmolarity, AtATM1 is relocalized within cells suggesting that AtATM1 may also have multiple functions in stress response and stress adaptation.

P0245. Expression patterns of *Arabidopsis thaliana* myosin promoter fusions with GUS reporter gene in transgenic plants

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Myosin proteins are eukaryotic actin- and ATP-dependent molecular motors. Myosins have been divided into at least eighteen classes. All the *Arabidopsis thaliana* myosins fall into two groups - class VIII and class XI. *Arabidopsis thaliana* encodes 17 putative myosins, four in class VIII and thirteen in class XI. Although myosins in animal cells have been studied in detail, little is known about the specific functions of myosins in plant cells. It has been suggested that class XI myosins are involved in cytoplasmic streaming while class VIII myosins are associated with the plasma membrane and plasmodesmata at the post-cytokinetic cell wall.

To determine the expression patterns of *Arabidopsis thaliana* myosins we have constructed several transgenes in which the reporter gene GUS is expressed under the control of different myosins promoter sequences. A part of myosin promoter-GUS transgenes show constitutive expression but in some cases the expression is tissue specific.

P0246. Mutations in a class XI and in a class VIII myosin genes affect the root hair growth in *Arabidopsis thaliana*

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Myosins are eukaryotic molecular motors moving along actin filaments. Reddy and Day (2001) described with in silico analysis that there are 17 possible sequences, which could code myosins in *Arabidopsis thaliana* genome. All 17 possible *Arabidopsis* myosins belong to two classes - class VIII containing 4 myosins and class XI containing 13 myosins.

The specific functions of *Arabidopsis* myosins are unclear. Here we describe phenotypes of homozygous T-DNA insertional mutants of two different myosin genes. Three different T-DNA insertional mutants of a same class XI myosin gene show similar but quantitatively different impaired root hair growth: length of root hairs of four-day-old mutant seedlings was 77, 50, and 41% of wild type root hairs, respectively. T-DNA insertional mutant of a class VIII myosin gene shows also decreased growth of root hairs with length of root hairs 55% of wild type root hairs.

It is known that plant tip growth is dependent on actin-based organelle/vesicle movement - thereby we suggest involvement of the class XI and class VIII myosins in cell elongation and tip growth. The specific roles of each myosin still remains to be resolved.

P0247. Identification of plant formin genes involved in the feeding site formation induced by root-knot nematodes

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Plant-parasitic nematodes *Meloidogyne* spp. induce an elaborate permanent feeding site characterized by the redifferentiation of root cells into multinucleate and hypertrophied giant cells. We have isolated by a promoter trap strategy an *Arabidopsis thaliana* formin gene, *AtFH6*, which is upregulated during giant cell formation. Formins are actin-nucleating proteins that stimulate *de novo* polymerization of actin filaments. We showed here that three type-I formins were upregulated in giant cells and that the *AtFH6* protein was anchored to the plasma membrane and uniformly distributed. Suppression of the budding defect of the *Saccharomyces cerevisiae bni1 bnr1* mutant showed that *AtFH6* regulates polarized growth by controlling the assembly of actin cables. Our results suggest that *AtFH6* might be involved in the isotropic growth of hypertrophied feeding cells via the reorganization of the actin cytoskeleton. Therefore, determining how plant parasitic nematodes modify root cells into giant cells represents an attractive system to identify genes that regulate cell growth and morphogenesis.

P0248. New evidence that 2,3-butanedione monoxime is not a general plant myosin inhibitor

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2,3-butanedione monoxime (BDM) is considered as a general inhibitor of myosin ATPase activity. BDM inhibitory effect has been used as evidence of myosin involvement in processes such as cell migration and focal adhesion in animal cells. However, some processes turned out to be insensitive to BDM in plant cells, e.g. cytoplasmic streaming in *Chara corallina* and light-dependent chloroplast repositioning in *Elodea* sp. These processes are known to be mediated by the actomyosin system. On the contrary, peroxisome motility in *Allium* and Golgi stack movements in *Nicotiana BY2* cells are inhibited by BDM. In the model plant *Arabidopsis thaliana* chloroplast photo-orientation movements are not based on cytoplasmic streaming. Nevertheless, actomyosin is an essential part of the chloroplast movement system. The aim of our study was to determine if BDM could be used in investigations of light-induced chloroplast responses in *Arabidopsis*. Using photometric method we found out that amplitudes and kinetics of accumulation and/or avoidance responses are unaffected by BDM. Summarizing, no general myosin inhibitor has been established so far, at least in plant cell studies.

P0249. The *Oryza sativa* Myosin XI B (*OsMyoXIB*) controls pollen development by photoperiod-sensitive protein localization

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We have identified an *OsMyoXIB* mutant in a phenotype screen of gene trap *Ds* insertion lines. This mutant showed male sterility under short day length conditions (SD) and fertility under long day length (LD). Under both SD and LD, the *OsMyoXIB* transcript was detected in all layers of anther wall, connective tissues, and young microspores. However, under SD, the *OsMyoXIB*-GUS fusion protein was localized only in the epidermal layer of anther tissues due to lack of 3'UTR and dilute domain sequences after *Ds* insertion. As a result, the mutant pollen development was affected thus exhibited male sterility. Under LD, the fusion protein was localized normally in all cell layers. Despite the normal localization, the protein was partially functional due to the lack of dilute domain, resulting in limited recovery of pollen fertility. Our results suggested that the photoperiod-sensitive expression of *OsMyoXIB* was controlled post-transcriptionally by 3'UTR and dilute domain sequences.

P0250. Phylogenetic origins of dioecy in *Silene*

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The aim of this project is to provide a phylogenetic/taxonomic framework for the evolution of dioecy within *Silene* (Caryophyllaceae). There are several dioecious taxa within the genus, traditionally classified in the sections *Elisanthe* and *Otites*. To understand how dioecy has evolved, these dioecious taxa need to be compared with their closest relatives. Available data suggest that the dioecious taxa within *Elisanthe* do not form a monophyletic group with the hermaphrodites in the section. Instead they seem more related to section *Conoimorpha*. This study is based on data from several *Silene* genes: (i) a gene sex-linked in the dioecious *Elisanthe* species (*SIXY1*) and a homologous autosomal gene in hermaphroditic taxa, (ii) intron sequences from the genes encoding the second largest subunits in the RNA polymerase gene family (*RPA2*, *RPB2*, *RPD2*) and (iii) chloroplast DNA. Remarkably, preliminary data suggest recombination between phylogenetically distant *SIXY1* lineages.

P0251. Adaptations to salinity in halophytic grass *Puccinellia limosa* Schur. (Holomb.)

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Seasonal changes of root and shoot ion content of halophytic grass *Puccinellia limosa* were monitored in the samples collected from its natural habitats, situated in the north Serbia during the vegetation season.

The total salt content of the soil ranged from 0.37% to 0.64%, whereas sodium concentration of the soil solution varied between 129 mmol and 218 mmol. Average content of some ions in the shoot, such as Na⁺ (127.5 ± 64.1 μmol g⁻¹ dry weight), Ca²⁺ (88.3 ± 46.5 μmol g⁻¹) and Mg²⁺ (61.1 ± 17.3 μmol g⁻¹) was lower comparing with the root, in difference to the content of potassium and, especially chloride whose concentrations in shoot were significantly higher. The Na/K ratio of the shoot was < 1, which is typical for halophytic monocots, and it is being increased along with enhanced Cl⁻ accumulation during the vegetation season, causing growth inhibition.

Ion retention in the root, high root capacity for ion accumulation, including calcium, low rate of salt movement to the shoot, as well as the doubled endoderm and mechanism of salt exclusion, are important features of *Puccinellia limosa*, limiting the deliver of ions to the photosynthetic tissues.

P0252. Structure and Histochemistry of the foliar laminae and vegetative propagation of *Phyllonoma laticuspis*, medicinal species.

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Phyllonoma laticuspis foliage is used by Mexican ethnic groups to heal mainly skin wounds like hurt and smallpox but also disease like diabetes and cancer. Chemical analysis of foliar laminae have demonstrated positive pharmacological activity. Histological and histochemical studies have been done to characterize the foliar laminae and propagation techniques through stakes and layers were applied for conservation. Foliar laminae were included in paraplant and they were dyeing for its observation. Stakes and layers were treated with indolbutyric acid (IBA) using different concentrations. Since the inflorescence arises from foliar laminae, structural characteristics were searched in order to distinguish if this organ is a stem or a leaf. Foliar laminae are dorsiventral and hypostomatic, presents tannins in the margin and peciolo and shows the entrance of three vascular beams in the base, elements that sign it has a leaf. The stakes didn't take root, while layers showed an 88% of rooting. Although *Phyllonoma laticuspis* is a hard species to take root, it could be propagated with the applied treatments and to benefit.

P0253. Drug accumulation by natural populations and cultivars of Cannabis and Ephedra species.

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It was studied natural populations 7 species of Ephedra, Cannabis ruderalis and 4 fibre cultivars of Cannabis sativa. The leaves samples of Ephedra were taken from different provinces, Cannabis ruderalis from Shu valley and south-east part of Kazakhstan. European varieties of Cannabis sativa cultivated in Shu valley. By the method of biochemical markers it was shown genetical differences between populations and species. Analysis of leaves composition revealed accumulation of tetrahydrocannabinol, ephedrine and their isomers. Separate isomers were identified quantitatively and qualitatively. Analysis of the populations and cross pollinated hybrids on a huge area give possibility to prove that drug accumulation is a genetical feature. Accumulation of separate isomers depends on hereditary peculiarities. Sometimes this is a dominant feature, sometimes codominant, but with a very active isoenzyme-synthase of this isomer (usually they have a common precursor). In this case several compounds are synthesizing simultaneously, but the substrate is one and activity of isoenzymes is different. As a result different amounts of isomers.

P0254. The cellular basis of interspecific differences in leaf size among C₃ grass species

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This study examined cellular mechanisms resulting in interspecific variation in leaf length among 16 C₃ grass species. The cell length profile of the mesophyll layer in the leaf growth zone was observed by Nomarsky interference microscopy, and various cell parameters related to division and elongation were calculated using a kinematic method. Leaf length showed a three-fold difference among the species. This difference in leaf length was mostly due to the difference in cell number and not to the difference in cell size. The difference in cell number among the species reflected the difference in cell division activity in leaf meristem, which was mostly determined by the number, not the rate, of cell cycle. Cell elongation processes showed close coordination with cell division activity. About 70 % of the variation in mesophyll cell size was determined only by the number of cells in the division zone. Species with high cell division activity had smaller mesophyll cells, which were caused by short cell elongation period.

P0255. Photoperiodic activation of protein kinase C expression in short-day plants

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Mechanism of flowering induction is up to this time incomprehensible. Wide spectrum of components of this induction does not exclude significant role in this transduction passway of photoperiodic signal through protein kinase systems. The aim of this work was to study protein kinase C (and) isoforms in leaves and stem of different short-day plants during photoperiodic induction. Among other components for alpha-protein kinase C

activation the significant place have Ca²⁺ ions, whenever process of eta-PKC activation is Ca²⁺ independent. Plants for investigation were short-day Nicotiana tabacum L. M. Mammoth, Perilla crispa and Chenopodium rubrum L. which obtained photoperiodic induction by 1, 2, 5, 10 and 15 short cycles (8 h of light and 16 h of darkness). PKC expression was studied by immunoblotting. As a result expression of 2 PKC isoforms in leaves and stem of plants were shown. Activation of Ca²⁺ dependent alpha-PKC during flowering induction signifies possible role of this protein kinase in floral signal transduction. The study of eta-PKC activity in plants is conducted at first and elucidation of this isoform role in flowering induction will be continued.

P0256. Seed morphology of Neotropical species of Bulbophyllum Thouars (Orchidaceae - Dendrobiinae)

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We examined the morphology of eighteen American species of the Pantropical orchid genus *Bulbophyllum*, covering all Neotropical sections, using SEM (Scanning Electron Microscopy). Seed morphology is quite uniform in shape and ornamentation, with seeds ovoid to fusiform, presenting long testa cells without inter-cell gaps and verrucose walls. Seeds of all species had at least one dichotomized wall and displayed a spiraled conformation. Seed size varies between 91 to 200 µm in length and 46 to 83 µm in width. Cell number is usually eight in the wide axis and one in the long axis. This study is the apparently the first to examine seeds of Neotropical species of *Bulbophyllum* by SEM and we found seeds much smaller than those reported in the literature for the subtribe, actually being one of smallest orchid seeds reported. We did not find seed characters that could be successfully used as diagnostic markers for taxonomic groups (new world sections *Didactyle*, *Xiphizusa*, *Micrantha* and *Bulbophyllaria* sensu Pabst & Dungs). Only within section *Bulbophyllaria* sensu Hamer & Garay seeds were distinct by presenting a larger size, even though they did not display differences in shape.

P0257. Maintenance the integrity of endemic and endangered species of Orchidaceae in Northeast Brazil - reproductive aspects

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We studied the isolation mechanisms in two pairs of endemic and endangered orchid species: *Cattleya elongata* / *C. tenuis* and *Sophranitis pfisteri* / *S. sincorana*. All species are pollinated by *Bombus brevivillus*: the *Cattleya* species by queens and the *Sophranitis* species by males and workers bees. The first pair presents strong floral similarity, floral synchrony, inter-compatibility and parapatric distribution, reproductive isolation being given by different habitats, as suggested by the occurrence of hybrids in disturbed areas. *S. pfisteri* and *S. sincorana* present dissimilar floral morphology, floral synchrony and sympatric distribution, reproductive isolation being given by partial inter-incompatibility and pre-pollination mechanisms: pollinarium deposition occurs on different places of the insects due to different length of the flower column - mesonotum for *S. pfisteri* and scutellum for *S. sincorana* - and inter-specific experimental crossings resulted in low fruit set and seed viability, preserving the genetic integrity of both species.

P0258. Morphological, physiological, and molecular biological characterization of a new thraustochytrid-like species, strain 12B that accumulates high levels of docosahexaenoic acid.

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Thraustochytrids are known for their high accumulation of omega-3 long chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA), a fatty acid which has been focused on its commercial use and physiological roles. We isolated one thraustochytrid-like microorganism (strain 12B) from a fallen leaf taken from estuary water of Okinawa, Japan. The DHA contents of strain 12B were up to 50% of the total fatty acid. Its productivity of DHA was 3.15 g/L/day at 30°C. Morphological analyses revealed that strain 12B possessed a unique ectoplasmic net structure, which has never described in any other thraustochytrid species. The electron microscopic observation of strain 12B showed that a spherical cell

was surrounded by multilayered cell walls. The cytoplasm filled with multiple oil globules and mitochondria. Mitochondria had tubular cristae. Some cells were multinucleate. The molecular phylogenetic analysis based on partial sequencing of the 18S rRNA gene showed that strain 12B was comparatively related to *Schizochytrium limacinum* SR21 and thraustochytrid A5-20. Thus it is considered that strain 12B is a new thraustochytrid species.

P0259. *Cuscuta jalapensis* parasite on *Coffea arabica* in Veracruz, México

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Cuscuta jalapensis has been detected parasiting coffee bean plants in Coatepec, Mexico. It is widely known that this parasite affects productivity of different crops. Coffee culture is quite important in Mexico so we decided to search how *Cuscuta* establishes in coffee plantations. How deeply goes the haustoria into the host. What about the enzymatic mechanism of penetration. We also studied the embryology of this plant; our goal was to study some aspects of the biology of *Cuscuta jalapensis* and its invasion strategies on coffee cultures. Samples in LRW resin were sliced 2µm width for light microscope observations; we observed haustoria at SEM and, we detected activity of peroxidase enzyme in 8µm cryostat slices, Sigma Immunochemicals technique. This orange filiform herb invades coffee plants through herbs and shrubs, on suitable hosts develops haustoria that penetrates beyond vascular bundles reaching medulla. We identify peroxidase enzyme involved in the generation of an entry pathway. We found a high incidence of abortion and observe perennation and autoparasitism. The only way to avoid infestation is to cut off infested plants and eliminate herbs aside

P0260. Light line in the seed coat of *Ipomoea purpurea* L. (Roth) Convolvulaceae

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The palisade layer of the seed coat is responsible for the water impermeability. *I. purpurea* and others Convolvulaceae have hard seeds because it is necessary mechanical scarification for their germination. In this paper, we describe the palisade cells development and their light line of *I. purpurea* seed coat, by light, confocal laser scanning microscopy and electron microscopy.

The youngest stages of the seed development have not light line. After the middle of the seed coat development it appears and the light line is more evident in the mature seed.

The palisade layer in *I. purpurea* is two or three stratified. The secondary cell wall of the palisade cells in *I. purpurea* is characterized by thickening of the radial cell walls.

Aniline blue dyed a band in the upper part of outer palisade cells then we consider that

light line is a callose deposition zone, among others compounds, running in a periclinal direction through the secondary cell wall.

P0261. The effect of plant water status on growth of olive fruit cells and tissues

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Studies were performed with olive plants, either container-grown or in the field, whereby the amount of water supplied was adjusted to induce transient or permanent water deficit during fruit development. Fruit volume, fresh weight, dry weights and transverse equatorial areas of the endocarp and mesocarp were measured at the completion of endocarp sclerification (8 weeks after full bloom, AFB) and at fruit maturity (21-22 weeks AFB). Cell size and cell number were determined with optical microscopy and computer image analysis. Water deficit during 4-9 weeks AFB, reaching pre-dawn leaf water potential (PLWP) of -3.1 MPa, reduced fruit fresh weight, fruit volume, and mesocarp transverse area at both stages, but endocarp area only at 8 weeks AFB. For moderate water deficit (-1.0 < PLWP < -3.2 MPa) imposed 8-19 weeks AFB, the mesocarp-to-endocarp ratio (fresh weight basis) was greater than that obtained under either well-watered (PLWP >

-1.1 MPa) or severe deficit conditions (1.8 < PLWP < -4.0 MPa). At the cellular level, water deficit appeared to reduce mesocarp cell size, and only occasionally and to a lesser extent mesocarp cell number.

P0262. Morfo histological comparison of edible fruits consumed in México of two species of cactaceae

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Stenocereus griseus and *Escontria chiotilla* are species of cactus producing edible fruits. In order to make a morpho histological comparison, samples of flowers and fruits were taken in different development stages and with them fresh and processed material were prepared. *E. chiotilla* has bracts and *S. griseus* thorns in the pericarpel. The flower of *S. griseus* has red petals and the unilocular ovary with numerous campilotropic ovules is larger than the *E. chiotilla*, ovary that is 2 cm in diameter and has yellow flowers in both species. The berry of *S. griseus* distinguishes by the presence of fibers between the pericarpel and the loculus. The pulp contains different proportions of betaxanthin and betacyanin pigments depending on fruit type while *E. chiotilla* flesh contains same proportion of pigments since it is a monotype. *S. griseus* seeds have esclerenchyma tissue in the seminal coat that confers hardness and a fluted and heavier cuticle than in *E. chiotilla* seeds. The development of seeds in *S. griseus* is asynchronous. Differences in the development process are described and quality parameters such as titratable acidity, total soluble solids and color are compared.

P0263. Phenology, pollination ecology and breeding system of *Polyalthia coffeoides* and *P. korinti* (Annonaceae)

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Comparatively little is known of the reproductive biology of palaeotropical Annonaceae. Populations of *Polyalthia coffeoides* and *P. korinti* have been studied in dry and intermediate zone forests of Sri Lanka. The petals separate early in development, but subsequently close to form a pollination chamber over the reproductive organs. Formation of the chamber is coincident with elevated internal temperatures and scent production over a two-day period. GC-MS analysis of the odour reveals the presence of volatiles that mimic beetle sex pheromones. The primary effective pollinator of both species is an *Endaeus* weevil (Coleoptera: Curculionidae), with pollen grains generally observed around the mouthparts and legs. The weevils are abundant and active around the flowers in the late evening and overnight. The flowers are protogynous, and studies of pollen/ovule ratios suggest that both species are facultatively xenogamous; this is corroborated by controlled pollination experiments, which reveal greater fruitset following cross-pollination than self-pollination. Variation in ISSR molecular markers indicates that both species have a mixed mating system.

P0264. The ultrastructural study of Shoot Apical meristem cells and their changes affected by Vitamin C in Faba bean

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Faba bean (*Fabaceae*) is annual herb and cultivated as vegetables. It has a great nutritional value and is considered as a rich source of protein in human's diet.

Ascorbic acid (vitamin C) may be found in all compartments of plant cells and participates in a several processes such as cell division and growth, etc.

In this study, seeds of Faba bean var. saraziri were cultivated in Dezful farm, Khozestan. Plants were treated by different concentrations of vitamin C (0-10-50-100-200 mg/L).

Meristemic samples were taken at vegetative and reproductive stages, and were studied by means of S.E.M. and T.E.M. microscopes.

The results showed that vitamin C caused changes in structure leading to meristem expansion and more flowers were produced through promoting meristematic mass cleavage and increasing

flower primordial. T.E.M. studies showed that the most changes were occurred in mitochondria, proplasts and cell walls. Increasing the number of nucleoli is one of the vitamin C molecular effects in these cells.

Keywords: apical meristem, vitamin C, Faba bean

P0265. Essential oil and secretory reservoirs in some *Hypericum* species

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The content of essential oil was assessed in *Hypericum perforatum* (0.3% d.w.), *H. elegans* (traces), *H. olympicum* (0.1% d.w.), *H. elatum* (traces) and *H. patulum* (traces). Location and ultrastructure of secretory reservoirs were microscopically examined in leaves, petals and stems. Reservoirs were formed in protophloem of veins and stem in every species examined, and in bushy species additional reservoirs were produced by stem cambium in secondary phloem. The mesophyll-located reservoirs spanned the whole thickness of leaf in *H. perforatum*, *H. elegans* and *H. olympicum*, while in *H. elatum* and *H. patulum* they were much smaller but more numerous. Generally, the ultrastructure of epithelial cells varied little between organs or species. The walls of epithelial cells facing reservoir cavity (that in every case could contain a mixture of electron-dense and electron-transparent secretion) were considerably loosened. Distinctive attribute of epithelial cells was presence of leucoplasts with homogenous stroma that in some organs was penetrated with a network of extensive but delicate invaginations of the envelope's inner membrane.

P0266. Morphological, developmental and anatomical differences between female and male form of nettle (*Urtica dioica* L.).

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Morphological, developmental and anatomical differences between female and male form of stinging nettle were investigated. Morphological differences concerned height of shoots, shape and size of leaves, number and location stinging hairs on leaf blade, and type of inflorescences. Shoots of male form were higher than those of female one. The leaf surface was also bigger in male form. More stinging hairs were observed on leaves of female form. The inflorescences of this form were short, close and decurrent, whereas those of male form, were dispersed and square with the shoots. Developmental differences concerned development and fall of leaves as well as appearance of inflorescences. Up to the period of blooming number of leaves in both investigated forms was similar. The inflorescences of male form appeared earlier than those of female form. Male and female forms of nettle didn't differ in respect of anatomy of investigated organs.

P0267. Analysis of the regulatory network controlling ovule identity in *Arabidopsis thaliana*

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Ovule development is a complex process, in which several important events occur: ovule identity determination, pattern formation and morphogenesis. Recently the ovule identity genes have been identified in *Arabidopsis thaliana*, which are three redundant transcription factors belonging to the MADS-box gene family: *SEEDSTICK* (*STK*), *SHATTERPROOF1* (*SHP1*) and *SHP2*. In the *stk*, *shp1*, *shp2* triple mutant the ovules are converted into carpelloid structures, whereas in *STK* ectopic expression the sepals are converted into carpelloid structures containing ovules. Several studies have shown that MADS-box transcription factor act through the formation of multimeric complexes. Also the MADS-box factors controlling ovule identity form these complexes. The assembly of these complexes are mediated by SEPALLATA MADS-box factors. The aim of the presented work is to identify the target genes of these ovule identity complexes.

P0268. The influence of ABA and GA₃ on activities of arginine and ornithine decarboxylases (ADC and ODC) and dormancy breaking of seeds of European beech (*Fagus sylvatica* L.).

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Dormancy is a feature of many seeds, especially those of forest tree species. The ripe seeds of European beech are in deep dormancy that requires for breaking a cold stratification (temperature 3°C and over 30% humidity). The correlation of arginine (ADC) and ornithine decarboxylases (ODC) activities and effect of abscisic acid (ABA) and gibberellin (GA₃) during dormancy breaking were determined. Whole nuts were treated with ABA (50 µmol) or GA₃ (100 µmol) and water as a control and were stratified at 3°C. GA₃ was found to stimulate activity of both enzymes and to break the seeds dormancy while ABA, in contrary, was found to act in opposite way. Our earlier studies on the mechanisms of dormancy breaking and metabolism of polyamines as dormancy regulators as well as the results presented here indicate that a possible involvement of both studied enzymes in a regulation of dormancy of European beech seeds.

P0269. Branches anatomy of *Fraxinus uhdei* (Wenzig) Lingelsheim and *Prunus persica* Batsch. infected by *Cladocolea* spp. (Mistletoe)

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True mistletoes are parasitic flowering plants. Seeds are disseminated by birds and deposited on host plants reducing their growth. Samples of 5 branches of *Fraxinus uhdei* and *Prunus persica* were collected in Mexico City. Blocks of 1 cm³ were obtained. Slides with typical cuts of the infected zone and the healthy zone were made. The wood of the two species has ring porous, their vessels are solitary and radial multiples, vessels pits are alternate. Its perforation plates are simple, rays have 1 to 4 cells and are heterogeneous and present fibre tracheids. The vessels in *P. persica* have helical thickenings and the outline is angular and the axial parenchyma is scanty diffuse. In *F. uhdei* the vessel outline is rounded and the axial parenchyma is of many types. The wood of mistletoe present diffuse porous. Vessels are solitary and clusters with the outline rounded, with pits alternate and simple perforation plates, present vascular tracheid. The axial parenchyma is in bands, present fibre tracheids. Rays are heterogeneous with 1 to 4 series. Both parenchyma types have abundant starch. The host anatomy is modifying when mistletoe penetrates, forming abundant contents.

P0270. Leaf Anatomy of the Turkish Endemic *Iris schachtii* Markgraf

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Iris schachtii Markgraf is an endemic Irano-Turanian plant species spreading in Central Anatolia, Turkey. Flower color shows variations within the species. In flowers bicolority is observed; yellow and purple, but these are not important for the separation of species into subspecies or variants. Leaf anatomy of the individuals having each color was examined. Leaves are linear-lanceolate and show parallel venation. Epidermal cells of unifacial ensiform leaves axially elongated and end walls are straight. Small papillae are present on epidermis. Stomata are amaryllis type. Stomata appeared under the epidermal cell layer. Epidermal cell numbers and stomata numbers per mm² of leaves are determined. Bundles of sclerenchyma cells occur at leaf margins and vascular bundles. Vascular bundles are lined in rows. Xylem of vascular bundles is directed towards the leaf center.

P0271. On seed vascularization in the family Pinaceae

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We have investigated the seed anatomy of over 70 species of 11 narrowly understood genera in the *Pinaceae* family. Our studies demonstrate the presence of two vascular bundle derivatives in the spermoderm of *Abies* Mill., *Cedrus* Trew, *Keteleeria* Carrière and *Pseudolarix* Gordon, located in the inner layers of mesotesta along

the ridges of the seed. Vascular bundles are developed in the seed coats of the representatives of different genera to various extents, and are composed of a varying number of elements (from 2-3 in *Abies religiosa* (Humb., Bonpl. & Kunth) Schlechtendal & Cham. and *Pseudolarix amabilis* (J. Nelson) Rehder to 15 in *Abies sibirica* Ledeb. and *Cedrus deodara* (Roxb.) G. Don in Loudon). The vascularization of the spermoderm of the mentioned genera, with the exception of *Pseudolarix*, had already been noted by M. P. Radais in the 19 century. However, in the majority of the more recent publications on the *Pinaceae* seed anatomy that we surveyed, these data were not taken into account or discussed. We are the first to describe the vascular bundles in the seed coat of *Pseudolarix*. Our discovery shows the importance of further research in this direction.

P0272. Ultrastructure of conidial ontogeny of *Septoria dictamni* Fckl. pathogenic to *Dictamnus angustifolius* G.Don.

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The ontogeny, delimitation, and release of conidia from conidiophores in pycnidium of *Septoria dictamni* were studied with light and transmission electron microscopy. Conidial ontogeny of *S. dictamni* was holoblastic and annellidic. Annellides arose from the inner cell layer of pycnidium. The primary conidia were simple holoblastic (without proliferations). They were produced as extensions from conidiogenous cells or as the swelled apex of the annellide, and projected into the central cavity of pycnidium. The next conidia were produced by percurrent proliferations. Some proliferations of the conidiogenous cells were sympodial. For every new conidium (excluding primary conidium), a completely new two-layered wall was formed. Annellations in *S. dictamni* were distinct. Delimiting septa associated with Woronin bodies were observed in the bases of each seceded conidia. Conidia in pycnidium of *S. dictamni* were liberated schizolytically.

P0273. Some anatomical and ultra-structural peculiarities in epigeoid, epilite, epixyl and epiphyt mosses from Ara mountain (Armenia)

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Some attempts for revealing adaptive peculiarities of structure for the mosses growing in the Ara mountain (the central part of Armenia, altitude - 1600 to 2570 m a. s. l., the *Quercus macranthera* shooting forest, mountain steppes, alpine meadows and other) were made. The study of leaves structure of the 58 species of mosses from few ecological groups (epigeoids, epilites, epixyls, epiphytes) by light microscope did not give desirable results. Later, the stem ultra-structure of the 15 species of mosses was studied by scanning microscope HITACHI S-405A. Vascular structure differentiation for the essential epigeoid species and absolute absence of such type of the tissues for the epilite mosses was revealed. For the representatives of remaining groups different levels of the vascular system development were registered. They compensate deficit of water by their capacity of absorbing it through surface. The prevalence of the last type of mosses species in Ara mountain is an evidence of its flora liability to xerophytisation.

P0274. Leaf anatomy of some species of *Tillandsia bulbosa* Hook. (Bromeliaceae) complex from Mexico

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Tillandsia is the largest genus of the Bromeliaceae with ca. 518 species distributed in tropical and subtropical America. This work presents the leaf anatomy of *T. balbisiana*, *T. bulbosa*, *T. butzii*, *T. caput-medusae*, *T. diguetii*, *T. seleriana* and *T. weberi*, species characterized by their sheaths forming a bulbous rosette and their blades involute. For each one of the species, samples of the middle third of the blade were fixed and processed according to the conventional histological techniques to obtain permanent anatomical slides. A comparative analysis was made. All taxa have peltate trichomes on the upper and lower leaf surface. The stomata are of the tetracytic type and are restricted to abaxial surface. The epidermal cells present undulated walls, thin cuticle, inner periclinal wall thickened and a silica body per cell. The mesophyll are differentiated in multiple layers of chlorenchyma and

water storage parenchyma. The vascular bundles are arranged in a single row and present a bundle sheath of fibers. Raphides were found in mesophyll cells. Also differences in the stomatic index, size of the vascular bundles and size of bundle sheaths were found

P0275. Germination of a Tropical Mistletoe, *Cladocolea loniceroides* (Van Tieghem) Kuijt Lorantheaceae

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This mistletoe grows as a hemiparasite on various trees that may have economic value for fruit, lumber or as ornamentals. The tree's physiology may be affected so severely that it eventually dies.

Many aspects of floral biology, germination and establishment have not been described for this plant. In this paper, we describe germination, penetration of the host, and establishment.

Samples of mature fruits germinating and developing on the host were obtained. Standard Microtechnique, light and Scanning Electron Microscopy (SEM) are used. We describe results with microphotography.

The fruit adheres to the host with viscin and mucilage, which originate in the middle layer of the fruit wall. A holdfast is formed during the germination. By means of haustorium and a penetration organ, the haustorium penetrates and becomes anchored in the xylem. A knowledge of these mechanisms of establishment may help devise methods of control of this mistletoe.

P0276. Anomalous grow in *Bassia sieversiana* (Pallas) W. A. Weber

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Bassia sieversiana is an herbaceous species belonging to Chenopodiaceae family which is known to present anomalous secondary thickness of the axial organs. In the stem the first cambium occurs early in ontogenesis. The succeeding cambia arise discontinuously, but subsequently they become circular. Before that, the initial cambium begins to produce thick walled and lignified ray cells in the phloem ring; these rays are linked with the xylem one and finally they include an area of secondary phloem. The succeeding cambium appears by dedifferentiation from parenchymatic cells of the internal cortex. The activity of the multiple cambia provides intraxylary phloem areas, disposed in concentric rings. It is thought that succeeding cambia can produce a thicker stem or root than a single cambium. In the analyzed material we have found a slow but constant decrease of the number and diameter of the xylem vessels resulted at the end of cambial activity; these increase in the first stage of new cambium activity. These features could be considered as an adaptative response to the interspecific competition.

P0277. Micromorphology and histochemistry of the glandular trichomes of *Cedronella canariensis* (L) Webb & Berth. from Madeira.

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Cedronella canariensis (L.) Webb & Berth. (= *C. triphylla* Moench, *Dracocephalum canariense* L.) is a sharp-smelling perennial herb which is endemic of Madeira, Azores and Canary Islands, being characteristic of the laurel wood. In this work, the indumentum of the leaves was studied by means of scanning electron and transmission electron microscopy and by histochemical methods. The indumentum possessed both non-glandular and glandular trichomes on both the abaxial and the adaxial surface of the leaves. On the abaxial surface, however, long non-glandular trichomes were present, which contrasted with the short, conic and sparsely distributed trichomes existing on the adaxial surface. Glandular trichomes of two types, capitate and peltate, occurred mainly on the abaxial surface of the leaves. Ultrastructural and histochemical studies of the secretory cells revealed that the secretion is lipophilic in nature, which agrees with our previous chemical study concerning to the isolated essential oils (Antunes et al. 1998).

Antunes T. et al. 1998. III Simpósio Fauna e Flora das Ilhas Atlânticas, Ponta Delgada, Açores.

P0278. Isolation and Identification of Crocetin Aglycons in *Crocus sativus* L. and Some Wild *Crocus* Species Stigmas by Chromatographic and Spectroscopic Methods

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Crocetin is a dicarboxylic 20-carbon carotenoid that occurs naturally mainly as glycosyl esters (Crocins) in *Crocus sativus* L. stigmas. Crocins are the main biologically active ingredients or the food coloring compounds of *Crocus* species stigmas. They are powerful quenchers of free radicals and could be used as anti-cancer agents. The main aim of the present study was to obtain and characterize of crocetin from commercial and some Iranian wild *Crocus* species stigmas by different available methods. Crocetin aglycons were isolated from dried methanolic extracts of stigma samples in basic and acidic solutions. The crystallized aglycons were dissolved in DMSO and passed through acetate cellulose filters. Finally, crocetins were qualitatively identified using analytical TLC and also an isocratic HPLC method and were characterized using UV-visible and NMR (¹H and ¹³C NMR) spectroscopies. All results and data that were obtained by the different methods confirmed the identity of the isolated aglycons and also showed that crocetin is probably the main aglycon of the carotenoid pigments (Crocins) in different *Crocus* species stigmas.

P0279. Reproductive modifications in *Phaseolus vulgaris* wild and cultivated varieties because of domestication.

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Mesoamerican and South American *Phaseolus vulgaris* possesses high potential for genetic improvements. In this investigation we compared some reproductive aspects of wild beans with their cultivated counterparts.

Buds were collected just before the anthesis and fixed with F.A.A. as well pods and seeds; included in JB 4 and finally cut at 2μ. The observation of the four genotypes revealed differences in the curvature angles of the nucellus, in the number of external tegument strata and the shape of the stigma. These include a "cushion" shape in wild types and a "brush" shape in cultivated types. The pollen was tricolporate, anguloaperturate and brevicolpate, as well as being covered by a pseudo operculum, contrary to what has been reported by other authors who describe it as only triplicate.

The transverse cut of the pods showed a higher concentration of tannins in the wild varieties in relation to the cultivated ones. The qualitative evaluation of the dominant color resulted in a dark seed for the wild varieties and a light seed for those that were cultivated. The wild varieties had less weight and size in relation to the cultivated varieties.

P0280. Comparative analysis of the sexual phase of nine species in the genus *Argyrochosma*, *Astrolepis*, *Cheilanthes* y *Pellaea* (Pteridaceae) in Mexico.

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A comparative analysis of the sexual phase of *Argyrochosma formosa*, *Astrolepis sinuata*, *Cheilanthes angustifolia*, *C. bonariensis*, *C. leucopoda*, *C. myriophylla*, *Pellaea atropurpurea*, *P. ribae* and *P. ternifolia* is presented. Fertile pinnae of each one of the species were collected in different states of Mexico, and were kept in paper bags until spores were shed. The spores were sown in Petri dishes containing agar supplemented with Thompson's. In all the species studies, spores are triletes, germination is Vittaria-type and prothallial development is Adiantum-type. The adult gametophytes of *A. formosa* are laminar-branched uniseriate filaments; those of *A. sinuata*, *C. angustifolia*, *C. bonariensis*, *C. leucopoda*, and *P. ternifolia* are laminar-branched to spatulate-cordiform with entire margins; those of *P. atropurpurea* are cordiform-reniform with entire margins, and those of *P. ribae* are cordiform-reniform with wide wings and irregular margins forming a thooted apex. No one of the species present trichomes. The sporophytes in *C. bonariensis*, *C. leucopoda*, *P. ribae* and *P.*

ternifolia are of sexual origin and in the other taxa are of apogamic origin.

P0281. Morpho-histological study in a Chilean specie *Centaurium cachenlahuen* (Mol.) Robinson.

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Morpho-histological study of stem and root of *Centaurium cachenlahuen* was carried out. *Centaurium cachenlahuen* belongs to the Gentianaceae. People of Chile has taken concentrate infusions for its hipertensive and sedative properties. Stem and root sectors were selected from several plants of this species. Permanent cross sections by dehydrating in an ethanol battery and preserved material in FAA (Formalin, Acetic Acid and Etilic Alcohol). Cross sections in Safranin -Fast -Green were stained. Cross sections of stem anatomy shows four wings described for the specie, an uniseriate and rectangular epidermal cells. These cells are isodiametric with convex walls covered by a thick cuticle, the cortex consists of two-three parenchyma layers with abundant and dense material. The vascular cylinder is enclosed by a sheath with a conspicuous fiber area. The cross section of the root shows irregular parenchyma cells in epidermis and cortex.

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P0282. Cloning and Expression Analysis of Genes Involved in Anthocyanin Biosynthesis from Asiatic Lily.

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Flower color is mainly derived from anthocyanin, one major class of flavonoids. We isolated cDNA clones encoding chalcone synthase (CHS), dihydroflavonol 4-reductase (DFR) and flavanone 3-hydroxylase (F3H), which are involved in anthocyanin biosynthetic pathway, from *Lilium speciosum* var. *rubrum*. The expression of these three genes was analyzed for *L. speciosum* var. *rubrum* (red tepals), *L. formosanum*, *L. x formolongi* (white tepals), and six varieties in Asiatic hybrid (red, white and yellow tepals). RT-PCR analysis showed that CHS, DFR and F3H transcripts were detected in red tepals, while weakly or not detected in white and yellow tepals. This indicates that the accumulation of anthocyanin is correlated with the expression of these three genes in Asiatic lily. We also analyzed the expression of three genes in *L. speciosum* var. *album* (white tepals), which is the variety of *L. speciosum* var. *rubrum* (red tepals). In this variety, CHS and F3H genes were expressed, but DFR transcript was not detected in white tepals, suggesting that lack of red pigments in *L. speciosum* var. *album* (white tepals) was correlated with the absence of the DFR transcript.

P0283. Fruit anatomy of *Amborella*

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Amborella trichopoda appeared as the basalmost extant angiosperm in recent molecular phylogenetic analyses. Earlier, based on general morphology, *Amborella* was placed in *Laurales*, thus, far apart from its present position. Also the structure of the fruits (fruitlets) seemed similar in *Amborella* and many *Laurales*. Developmental investigation of pericarp anatomy in *Amborella* showed its differentiation into three histogenetic zones (composed of five histological ones): exocarp (derived from outer epidermis), mesocarp (derived from mesophyll) (three zones: 25-35 layers of parenchymatic cells without idioblasts, 6-18 layers of thick-walled, unligified, canalculated sclereids, and 1 layer of thin-walled cells), and endocarp (derived from inner epidermis) (2-4 layers of small sclereids with thick, unligified walls). The histological differentiation of histogenetic zones of the pericarp in *Amborella* differs from that in *Lauraceae* and *Monimiaceae* s.l., in which a lignified putamen is formed by 1-2 layers of specialized cells of endocarp. Thus, fruit anatomy of *Amborella* is unique.

P0284. Green plastids, as judged from their chlorophyll auto-fluorescence, and starch distribution in internal stem tissues of three Mediterranean woody species during the year

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The occurrence and distribution of green plastids in internal stem tissues, i.e. cortex, wood ray parenchyma and pith cells, was examined with epi-fluorescence microscopy. Green plastids in deep stem tissues are supposed to help toward respiratory CO₂ recycle. Starch accumulation was also investigated. Three species having adopted different life strategies were studied. Pith and wood parenchyma cells of *Euphorbia acanthothamnus*, a drought deciduous species, show chlorophyll fluorescence throughout the year, though during the leafless summer period fluorescence level as well as starch content seem to be relatively low. Chlorophyll auto-fluorescence in internal tissues of the winter deciduous species *Platanus orientalis* increases in summer during leaf bursting, reaching its maximum before leaves drop. In the same period starch content is high. Chlorophyll fluorescence in the evergreen sclerophyll *Nerium oleander* deep stem tissues follows different pattern: it remains high and constant throughout the year. By contrast, starch is accumulated during rainy season.

P0285. Leaf histogenesis in *Buxus sempervirens* L.

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Immature and mature leaves (from first and second years) of *Buxus sempervirens* were observed in order to emphasize the histo-anatomical peculiarities related to their persistency. The foliar primordia have a semilunar shape determined by an intense activity of the abaxial meristem in the first stages of development. The marginal growth of the leaves is of median - submarginal type. The mesophyll is compact, dorsiventral, hypostomatic. At the young leaves, the palisade parenchyma consist in 2-3 layers of isodiametric cells which become elongated in mature leaves, especially in the second year. The vascular bundles present a girdle of sclerenchyma obviously more developed in the xylemic pole, contrary with the general case, when it is developed only or especially in the phloemic pole. The 2 years old leaf present isolate strands of sclerenchyma unconnected with the conducting tissue and two thick strands at the leaf edges. The diameter of the xylem vessels is very small. The stomata are localized at the same level with the epidermic cells, but the guard cells exhibit two extended crests which delimit a cavity.

P0286. Microscopic surgeries on meristemic parts and the place of archegonium in prothalli of *Adiantum capillus - veneris*

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Spores of *Adiantum capillus -veneris* give rise to a heart shaped prothalli after 40 days of their germination.. Microscopic surgeries are done on two parts of prothalli: sexual notch, the place which archegonium are produced. For the first study prothalli with completed notch were collected and their meristemic part and sexual notch were separated. For the second study, 40 days old prothalli were collected and with a very sharp pin the area which archegonium will be differentiated in, were destroyed. The prothalli of first group after starting their growth produce many prothalli on the sides of themselves. These new prothalli grow and become heart shaped with normal sexual organs. After fertilization sporophytes were seen on these prothalli. This is like the growth of lateral buds in advanced plants after removing the apical dominance.

After 10 days the second group of prothalli produces a very huge cellular mass which is very similar to the calluse of advanced plants. On this calluse first protonema and then prothalli are produced. The prothalli become heart shaped and make sexual organs. After fertilization sporophytes can be seen on these prothalli as the usual ones.

P0287. Anatomical structure of Scots pine wood depending on its geographical origin and temperature factor.

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The purpose of the work is the study of anatomic features of tree ring structure of Scots pine. This research was conducted for different climatotypes with different geographical origin and climatic characteristics growing in the Boguchany provenance trial. According to climatic data of Boguchany weather station for the research was selected two years with different temperature of vegetation period. The study of the number of cells in row, cell diameter, cell wall thickness was made with the help of Image-system and special computer programs. For each climatotype were selected 5 core samples. Climatotypes are from north and south taiga and from south Zabaikalie with moisture deficit. The mean cell diameter of cold vegetation period is significantly different from warm for all climatotypes. The increase of small cells percentage is in cold vegetation period, for the pine from north taiga the increase is smaller. However, the pine from north taiga has smaller cell diameter and cell wall thickness. The cell wall thickness has a lesser response to vegetation period temperature.

P0288. Pericycle and endodermis in the root, stem and leaf of monocotyledons

V. G. Alves¹, G. Melo-de-Pinna¹, R. C. Arruda², E. Scremin-Dias³, D. C. Silva⁴, C. G. Silva¹, N. L. Menezes¹;
¹Universidade de São Paulo, São Paulo, Brazil, ²Universidade do Rio de Janeiro, Rio de Janeiro, Brazil, ³Universidade Federal do Mato Grosso do Sul, Mato Grosso do Sul, Brazil, ⁴Universidade Estadual de Santa Cruz, Bahia, Brazil.

Most researchers attribute primary thickening in monocots stems to the PTM (Primary Thickening Meristem). This paper shows that, in species of *Asparagus*, *Bulbine*, *Canna*, *Cephalostemon*, *Chlorophytum*, *Echinodorus*, *Cipura*, *Ctenathe*, *Curcuma*, *Cyperus*, *Hypoxis*, *Kaempferia*, *Lagenocarpus*, *Reineckia*, *Rhoeo*, *Ruscus*, *Sansevieria*, *Scleria* and *Xyris*, the of procambium cells sequence, the pericycle forms vascular tissue and adventitious roots throughout the life of the plant. The endodermis is the innermost layer of the cortex with Casparian strips, starch grains, crystals phenolic compounds or suberin lamellae. The endodermic meristematic activity, observed in the roots of all species examined, gives rise to part of the cortex. In the stem and the leaf, the endodermis may (or may not) have meristematic activity, giving origin to part of the cortex (stem) or part of the mesophyll (leaf). In the shoot apex, the pericycle and the endodermis assemblage seems to be only one meristem, giving origin to the vascular tissues and cortical cells, respectively.

P0289. Morphogenetic Study of *Rhynchosytilis coelestis* Rchb.f. ex Veitch In Vitro

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Green pot seeds of *Rhynchosytilis coelestis* (Rchb.f.)Rchb.f. ex Veitch were culture on MS medium supplemented with 20% coconut water and 5% tomato juice for 1,2,3 and 4 months. The protocorm-like bodies and seedlings had been observed by scanning electron microscope. First of all, an oval shape embryo became swollen and released from seed coat within 1-2 months. After that, the embryo had concave top end and the opposite end was sharp. The globular embryo changed to be heart shape and torpedo-like, respectively. The concave end was subsequently convex at the top. After two month of incubation, leaf was formed. No tap root was formed. Adventitious root formation occurred in four months. Rhizoids, root hair- like structures, were seen early on the embryo surfaces and grew up until the adventitious root was formed. Stomatal apparatus was also seen in an early stage of the development of the embryo.

P0290. Effects of Ethanol, Methanol on the Structure and Ultrastructure of the Vegetative Meristem, Formation of Flower Components and Useful Vase Life in *Dianthus Caryophyllus* L.

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The one-year-old cuttings of *Dianthus* planted. They were given alcoholic treatments with 3, 6 and 9 percent concentrations. Alcoholic treatments lead to the meristem assumes the features of a generative meristem. The greatest effect of alcohol was in alcoholic treatments with 3%, particularly ethanol. The number of flowering shoots and flowers increased, and accelerated the flowering period.

In the investigations by T.E.M, chromocenters were determined in addition to observation of anticlinal and periclinal divisions in tunica cells, corpus, increase in thickness of cell walls, increase in nucleus volume and density of nucleic substances, as well as expansion of the vacuole system. The study buds flower with same of size and age in control and treated plants were shown that alcohol caused acceleration ontogeny, in flower organs. Ethanol and methanol 3 percent raised number of shoots flowering and flower.

Alcohol treatments on cut flower with the same size of peduncle showed that lower concentration delay aging and drooping in flowers. Increased of useful life in cut flowers were more visible in ethanol.

P0291. Anatomical alterations in pigeon pea roots under soil aluminium toxicity.

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The objective of this study was to determine the effects of aluminium upon the root anatomy of two pigeon pea cultivars, IAPAR 43-Aratã and IAC Fava Larga, and provide evidence of the differential tolerance to aluminium between these cultivars. Four days after sowing in sand, plants were transplanted to plastic boxes with 0% and 58% soil aluminium saturations, in a growth room under irradiance of $89.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, photoperiod of 8 hours and temperature of 29°C. A completely randomized design in a factorial scheme 2x2 with four replicates were used. Twenty days later, samples were fixed in FAA, the cross sections taken from paraffin-embedded material, stained with safranina-fast green in toluidine blue, and mounted in Canada balsam. Moreover, samples of living material were cut with freezing microtome and stained after dehydration, and photographed with a photomicroscope. Anatomical alterations in root apical regions of both cultivars evidenced that under soil aluminium toxicity a tissue disorganization takes place. However, since damage effects were similar, it was not possible to distinguish aluminium tolerance between cultivars. (Fapesp 01/03597-0)

P0292. PEACE, a MYB-related transcription factor, regulating pigmentation in flowering peach, can alter morphological characters of transgenic tobacco

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It is well known that plants use many MYB transcription factors to regulate the secondary metabolism and morphogenesis. One MYB-related gene obtained from pink petals of flowering peach (*Prunus persica*) can induce anthocyanin biosynthesis in colourless sectors of variegated petals. This gene was named PEACE (peach anthocyanin colouration enhancement). In this study we examined PEACE function by generating transgenic tobacco plants via *Agrobacterium* mediated transformation. About 40 plants were regenerated. Seventeen plants out of a total of 24 were confirmed as harbouring the PEACE gene by PCR. Divergent morphological changes were observed in transgenic plants expressing PEACE compared to control tobacco plants treated with wild *Agrobacterium* strain LBA4404 without PEACE, e.g. shorter plant height, paler flower colour, shorter flower length, petals bending outward and small white lesions on the leaves. Also lower seed fertility and higher chlorophyll content was observed occasionally. Genetic analysis of the progeny will reveal the molecular mechanism of morphological and physiological alterations caused by the PEACE gene.

P0293. Pericycle and Endodermis in Monocotyledons

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¹USP, São Paulo, Brazil, ²Universidade do Rio de Janeiro, Rio de Janeiro, Brazil, ³Universidade Estadual de Santa Cruz, Bahia, Brazil, ⁴Universidade Federal do Mato Grosso do Sul, Mato Grosso do Sul, Brazil.

Most researches attribute primary thickening in monocots stems to the PTM (Primary Thickening Meristem). This paper shows, in species of *Asparagus*, *Bulbine*, *Canna*, *Cephalostemon*, *Chlorophyton*, *Echinodorus*, *Cipura*, *Ctenathe*, *Curcuma*, *Cyperus*, *Hypoxis*, *Kaempferia*, *Lagenocarpus*, *Reineckia*, *Rheo*, *Ruscus*, *Sansevieria*, *Scleria* and *Xyris*, that in the procambium cells sequence, the pericycle forms vascular tissue and adventitious

roots throughout the life of the plant. The endodermis is the innermost layer of the cortex with Casparian strips, starch grains (starch sheath), crystals or suberin lamellae. The endodermic meristematic activity was observed in all species examined, which give rise to the innermost layer of the cortex in the root, stem and the innermost layer of the leaf mesophyll. In the shoot apex, the pericycle and the endodermis appear to form a single meristem, giving origin to the vascular tissues and cortical cells, respectively.

P0294. Companion cell and vein type in alfalfa source leaves

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In *Medicago sativa* blade, companion cells present transfer cell features. In source leaves, that support plant growth, structural differences have been observed between minor and main veins that are involved in assimilate loading and unloading respectively. In minor veins, transfer cells are three times larger than sieve tubes and wall ingrowths are finger-like, being always more developed in the old leaves. The presence of transfer cells in these minor veins has been correlated with apoplasmic mineral nutrient cycling, that is closely associated with carbohydrates transport from source to sinks. In the main veins, companion cells and sieve tubes exhibit the same diameter, wall ingrowths are papillate and their area is half to third as that of transfer cells in minor veins. Moreover, early stages of these transfer cell differentiation have been observed in these main veins that contain a procambial area. Structural differences observed in minor and main veins are discussed in relation with mechanisms of assimilate compartmentation.

P0295. Exogenous auxin affects formation of discrete vascular strands in *Arabidopsis* mutant *pin1*

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Organogenesis and vascular differentiation are interrelated processes controlled by the polar auxin transport (PAT). Mutation of the *PIN1* gene, regulating PAT in a shoot, is supposed to inhibit these processes. However, in *pin1* mutant organ formation is arrested only in early stages of a generative shoot development. Vascular differentiation is not inhibited, although vascular tissues do not form discrete bundles.

Analysis of ontogenetic changes in the mutant vascular pattern shows that initial syphonostelic system changes into eustele and vice versa few times in the same shoot. Eustele formation is correlated with the initiation of bulges and deformed organs. Eustele formation can be induced in the early ontogenetic stages by exogenous auxin.

At the site of IAA application, primordia are initiated and the vascular system forms quite discrete bundles. Above the primordium initiation level, syphonostelic system returns. IAA affects then organogenesis and vascular strand separation. In young shoots of *pin1* only exogenous auxin may induce both processes, while in older shoots they occur spontaneously.

P0296. Developmental changes in several isoenzyme systems during somatic embryogenesis of *Petunia x hybrida*

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The present study reports on the expression of some isoenzymes during somatic embryogenesis process in *Petunia x hybrida* callus cultures. The presence of 2,4-D, the concentration (1mg/l) and the time of cultivation (5 days) had a determinant role in embryonic competence induction. Competent cells expressed this embryonic potential by their subcultivation on culture medium without hormones. The activity of some enzymes as peroxidases (POX), glutamatoxaloacetate transferases (GOT), non-specific esterases (EST) and acid phosphatases (PAC), was monitored during progressive stages of the somatic embryogenesis. Moreover, specific protein and isoenzyme patterns of POX, GOT, EST and PAC in induction and expression phases of embryonic competence were analyzed to identify some embryonic markers. The variations observed in activity and electrophoretic spectra of these enzymes at different stages of culture emphasized the physiological or biochemical changes underlying the process of differentiation and were important for the establishment of the tissue specific biochemical characteristics that were expressed and maintained in cell culture.

P0297. The phenomenon of vivipary in gymnosperms and angiosperms.

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The vivipary is the especial type of propagation, when the generative or vegetative diaspore forms the seedling (propagula) just on the maternal plant without the dormancy period. The place of the vegetative diaspores formation varies (inflorescence, leaf, shoot or root). The vivipary could be either obligate or facultative. As the comparative analysis of vivipary distribution revealed, it is inherent mainly to the angiosperms (173 species of 43 fam.) and ferns (197 species of 8 fam.). The vivipary occurs quite rarely among the gymnosperms. In *Gnetum gnemon* in the greenhouse of BIN RAS the propagulas were found on the leaves of lower part of the crown. The propagulas aroused endogenously on the upper surface of the leaf's apical part (average 2.60 ± 0.68 per leaf), their stage of development varied from the groups of meristematic cells up to the shoot with two pairs of leaves. The leaves bearing the propagulas were in average twice smaller than another ones at the same plant. Probably, the facultative foliar vivipary in *G. gnemon* is the response to the stressing factor (long temperature fall). The ability of plant to the vivipary is the reserve of reproductive systems.

P0298. Ontogenetic transition from protostele to dictyostele in *Ceratopteris richardii*: Cell fate mapping reveals correlations with shoot growth

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The remarkably regular cell division pattern in juvenile and adult shoot apices of *Ceratopteris richardii* facilitates mapping of cell lineages and cell fates in successive derivative segments of the shoot apical cell (AC), from embryo to mature sporophyte. The developmental transition from protostele to dictyostele is autoapomorphic in ferns. The origin of leaf ACs (LACs), leaf primordia (LPs) initiation and early development, bidirectional procambium differentiation, and the simultaneous origin of LACs and root ACs (RACs) were unaltered during ontogeny. The switch from procambial to parenchymatous fate by the inner cells of the adult shoot apex, which is the basis of changing stelar type, was correlated with alteration in timing of the onset of cell proliferation in incipient LP; it was delayed from the third segment (S_3) to S_{10} . Correlated with this were changes in shoot apical meristem structure, alteration in rhythm of LP development from successive to almost simultaneous development of three successive LPs, and changing leaf shape and leaf trace structure.

P0299. Male flower anther wall development in *Dioscorea nipponica* Makino (Dioscoreaceae)

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Some regularities of anther wall formation and their correlations with sporo- and gametophyte processes in *Dioscorea nipponica* (Dioscoreaceae) were revealed. *Dioscorea* species is known to have 4-loculed anther. For the first time it was founded out the succession of laying down the anther wall initials, and wall development from the connective side till the stage of mature pollen grains. The anther wall formation is realized according the centripetal type, but with different variations on the distal and dorsal sides. The tapetum cells occur as the result of parietal cells division. Besides, these parietal cells also are of different origin, different form and moment of origin. The tapetum consists of secretory binuclear cells, without reorganisations. Maximum of its development tapetum cells achieve at the stage of nonvacuolated microspores; its programmed cell death occur in the end of differentiated mitosis in vacuolated microspore. Mature anther wall (distal side) consist of 2 lays: epidermis and endothecium, dorsal side - multilayer endothecium.

P0300. Overexpression of the *Arabidopsis thaliana* MADS-box gene AGL21 confers leaf-like characteristics to floral organs.

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AGL21 is a member of the MADS-box gene family of transcription factors. In order to unravel the function of AGL21 during

Arabidopsis thaliana development we characterized loss of function and gain of function lines for AGL21. While the former did not present any obvious phenotypic alterations the overexpression lines (35S:AGL21) showed altered behaviour during flower transition and flower development. The sepals in 35S:AGL21 lines sepals are more elongated than wild type sepals and they have stellate trichomes in both abaxial and adaxial surfaces. The petals of 35S:AGL21 lines are greener and significantly larger than in wild type plants. Carpels of these lines also bear stellate trichomes in contrast to wild type carpels. Under Scanning Electron Microscopy the petal and sepal cells of overexpression lines are flat rather than conical as in wild type and leaf-like respectively. These results suggest that AGL21 ectopic expression interferes with *Arabidopsis* normal floral development.

P0301. On the so-called "hydrocyte system"

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The formation of hydrocyte system is rather often takes place in seed plants, especially in flowering plants. In particular, it is discovered when formation the root shoots from the adventitious buds in the rootstock plants; when natural knitting of roots, and when different methods of shoot grafting. More strikingly is becomes apparent during the regeneration both in vivo and in vitro. The intensity of its development mainly depends on the organ's morphological nature, its functional loading and to an even the lesser degree depends on the taxonomical affiliation of object. More detailed analysis of hydrocyte system both at the light and electronic microscope levels showed its complicated histological structure. It includes the water-conductive elements (hydrocytes-tracheids, rarely - the vessel members), the phloem elements (the sieve cells and the sieve tube members with companion cells) and tracheidal parenchyma cells. The latter accomplish functions of transferring, supporting, accumulation and storing of reserve substances. Therefore, the so-called "hydrocyte system" can be regarded as original variety of seed plant conductive system.

P0302. Structural aspects of the cold and warm stratification effects to embryo development in seeds with complex deep morpho-physiological dormancy

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The structural responses of embryo cells to the conditions of warm and cold stratifications were investigated in *Tulipa tarda* seeds during embryo development. It was shown that as early as 24 h after imbibition, the structural changes in nucleus, chondriom and plastidom of embryo cotyledon epidermal cells (EC) testified the metabolism activation. After 72 h of stratification the structure of EC in the warm and cold conditions were similar indicated an active metabolic processes related to both transport to cotyledon from embryonal cavity and mobilization of their own reserves. It was concluded that initial stages of *T. tarda* embryo development has been started after achievement of certain level of water imbibition. The growth of the embryo ceased gradually after 20 d of warm stratification. Only in the cold the embryo development was fully completed after 52-62 d when EC were characterized by strong multiplication of protein synthesizing apparatus. The cells of shoot and root apical meristem had no any structural changes during all stages of embryo development. The mechanisms of low temperature effects to embryo development were discussed. RFBR 03-04-49494.

P0303. The role of smooth endoplasmic reticulum (SER) in regulation of developmental rhythms in early-spring ephemerals

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The tubular SER structure and surface density of its membranes in ground tissue cells of corm bulb in *Scilla sibirica* were investigated at different stages of annual growth cycle. In autumn and winter the degree of SER development was low. In early spring the surface density of its membranes per cell increased and SER usually appeared in the form of single tubules. In summer the SER proliferated greatly. In addition to single elements, twisted tubules in the form of compact network were observed. In these case the degree of SER density was similar to that in secretory cells. The

AER is known to be an organelle that is involved in terpenoid biosynthesis including sesquiterpenes. The abscisic acid, growth-inhibiting hormone responsible for seed and bud dormancy, belong to the category of terpenoids. Thus, it appears that summer dormancy of bulbs in *Scilla sibirica* is closely related to the development of SER in corm that is involved in abscisic acid biosynthesis inhibiting growth processes. The financial support of Russian Foundation Basic Research is acknowledged (project 03-04-49494).

0304. Ultrastructural Aspects Of Oil Gland Initiation And Specialisation In *Citrus limon* L. Ovary

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Ontogeny of the essential oil glands and ultrastructural features associated with their specialisation have been investigated in *Citrus limon* L. ovary wall during flower and fruit development. Oil glands initiate in young floral buds and attain their maximum number in green fruit peel. Five ontogenic stages are recognized, based on the histological aspect of the secretory structure. Oil glands originate from a pair of meristematic cells which later give rise to a globular main part and a stalk. The young gland consists of a central group of compact polyhedral cells, rich in ribosomes and plasids, and peripheral layers of flattened cells. After completion of the divisions, a single cell in the center of the gland demonstrates an increase in electron density, degenerated organelles and disintegrated membranes. The cytolysis extends centrifugally until an inner cavity is formed surrounded by cells in active secretion. Terpenoids seem to be synthesized in plastid matrix, transported through ER elements, and poured into the central cavity after fusion of the latter with the plasmalemma. Peripheral cells are modified into a protective sheath

P0305. Regulation of the V-ATPase

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V-ATPases are universal energizers of eukaryotic membrane systems and belong to the most fundamental enzymes in nature. Their primary function, the acidification of endomembrane compartments, makes them essential for a variety of cellular processes including secondary active transport, protein targeting, vesicle trafficking and membrane fusion. Because of their essential and complex functions it is important to determine the mechanisms that regulate the activity of these proton pumps. The genome of *Arabidopsis* contains 27 VHA-genes encoding the 13 different subunits necessary to form active V-ATPase complexes, thus offering an enormous potential to form different V-ATPase isoforms. Using reporter genes, we have identified tissue-, organell- and stress-specific isoforms and will present our approaches to dissect their biological functions. Furthermore we show that VHA-C is phosphorylated by a novel protein kinase and contains a nucleotide-binding site and will present our attempts to characterize the in vivo function of these regulatory mechanisms.

P0306. The autophagy-associated Atg8 gene family operates both under favourable growth conditions and under starvation stresses in arabidopsis plants

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Arabidopsis plants possess family of nine *AtAtg8* gene homologues of the yeast autophagy associated *Atg8* gene. To gain insight into these genes functions in plants, we transformed *Arabidopsis* plants with chimeric genes encoding two *Atg8* proteins fused to N-terminal GFP and C-terminal HA (hemagglutinin) epitope tags. Analysis of these plants showed that under favourable growth conditions, the *Atg8* proteins are efficiently processed and subsequently degraded. These proteins were localized to autophagosome-resembling structures both in the cytosol and in the central vacuole in a similar manner to their processing and localization under starvation stresses. Treatment with a cocktail of proteasome inhibitors did not prevent the degradation of these proteins, implying that their degradation takes place in the vacuoles as is occurring in yeast. Our results suggest that in plants the cellular processes involving the *Atg8* genes function efficiently in young, non-senescent tissues both under favourable growth conditions and under starvation stresses.

P0307. Gravisensitivity of plant meristematic cells under clinorotation

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Data of clinorotation influence on mitotic index and DNA content in meristem root cells of 3-5-7 day pea seedlings were occurred. Structural-functional organization as well localization of cytoskeleton actin filaments in root and marginal meristem cells under clinorotation demonstrated the primary mechanisms of meristem cell gravisensitivity. Destruction degree of meristem organization depended from clinorotation period. Under clinorotation, the nucleolus functional activity as well mitotic index was inhibited in the early stages of seedling development. Contradictory, the growth of marginal and root meristem cells intensified. The stress influence of clinorotation on the early stages of plant development was determined as a level of summary protein synthesis and hsp70 presence in pea seedlings. The results allow to necessary information for future investigation on molecular basis of cell cycle regulation. Determination of meristem cell changes furthers both the prognosis of experimental plant cell specialization and to work out methods for correction of the alterations in space plant technologies.

P0308. Effects of hypergravity environment on lignin formation in Arabidopsis

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Lignin, forming an important chemical modification of secondary cell walls, imparts mechanical strength to plant bodies. Deposition of lignin to secondary walls was essential during evolution of land plants. In this study, effects of hypergravity environment on lignin deposition in shoots of *Arabidopsis thaliana* was examined. In seedlings grown for 3 days after a hypergravity treatment for 24 hours, xylem vessels in the basal region of the shoots showed intense staining with phloroglucinol-HCl and pronounced autofluorescence under UV light. Lignin contents, quantified by acetyl bromide, were slightly increased in secondary walls of shoots exposed to hypergravity. The effect of hypergravity on lignin deposition in secondary walls of shoots was partially reduced in the presence of gadolinium, which is a blocker of mechanoreceptors. Our results suggest that lignin formation is positively regulated under hypergravity, and mechanoreceptors were partially involved in the positive regulation of lignin formation under hypergravity.

P0309. Expression of CsPIN auxin efflux carrier genes during gravity-regulated peg formation of cucumber seedlings

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When cucumber seeds germinate in a horizontal position, seedlings form a peg on the lower side of the transition zone. We have shown that a threshold level of auxin is required for peg formation. We have also shown that cucumber seedlings in microgravity develop a peg on each side of the transition zone, suggesting that gravistimulation decreases auxin and suppresses peg formation on the upper side of the transition zone on the ground. It is hypothesized that modification of auxin transport due to gravity causes the reduction of auxin on the peg-suppressed side of the transition zone. To verify this hypothesis, we isolated 6 cucumber cDNAs of *PIN* auxin efflux carrier genes and investigated their mRNA accumulation in the transition zone by *in situ* hybridization. Our results show that mRNA of *CsPIN1* and *CsPIN6* accumulates in several tissues including endodermal cells that are predicted to sense gravity. Furthermore, clinorotated seedlings of cucumber increase mRNA of *CsPIN1* and *CsPIN6*. These results suggest that *CsPIN1* and *CsPIN6* could play a role in gravity-response to regulate peg formation of cucumber seedlings.

P0310. PnSCR, a gene responsible for shoot circumnutation in Pharbitis nil

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An agravitropic mutant of morning glory (*Pharbitis nil*), *weeping*, has defect in shoot gravitropism. Our previous results showed that *weeping* were also defective in proper endodermis development and in shoot circumnutation. Such defects in circumnutation were also observed in endodermis-less *Arabidopsis* mutant, *scarecrow* (*scr*). In search of the mutated gene of *weeping*, cDNAs for *SCR* homolog from morning glory (*PnSCR*) were cloned from both wild type and *weeping*. We found three nucleotides insertion in *PnSCR* obtained from *weeping*. To investigate whether this mutation causes a loss of function of *PnSCR*, we performed complementation test by introducing either wild type *PnSCR* or *weeping* type *PnSCR*, respectively, into *Arabidopsis scr* mutants. As a result, none of the defects were rescued by *weeping* type *PnSCR*, whereas wild type *PnSCR* rescued them. This result indicates that defects in gravitropism, proper endodermis development, and circumnutation observed in *weeping* are attributable to the loss of function of *PnSCR*.

P0311. The role of auxin and ethylene for gravitropic differential growth of coleoptiles and roots of rye- and maize seedlings

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According to the Cholodny-Went hypothesis differential growth is brought about by the redistribution of auxin (indolyl-3-acetic acid, IAA). We studied the relevance of auxin and ethylene for gravitropic differential growth in shoots and roots of rye- and maize seedlings. As earlier demonstrated, incubation of coleoptiles in dichlorophenoxy acetic acid (2,4-D) resulted in a two- to threefold length increase compared to water controls. In spite of this effect on growth, gravi-curvature was similar to water controls. In contrast, inhibition of ethylene synthesis prevented differential growth of coleoptiles and of roots. Inhibition of ethylene perception in horizontally stimulated maize roots growing on surfaces inhibited the roots to adapt growth to the surface, resulting in a vertical orientation of the root tips. This effect is accompanied by up- and down-regulation of a number of proteins as detected with the 2D-MALDI-TOF. Together the data challenge the regulatory relevance of IAA-redistribution for gravitropic differential growth. They corroborate the crucial regulatory relevance of ethylene for gravitropic growth, in both roots and coleoptiles.

P0312. Microgravity-specific changes in gene expression after short-term exposure of *Arabidopsis thaliana* cell cultures

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Cell cultures of *Arabidopsis thaliana* (cv. Columbia) were used to screen for early alterations in gene expression as a response to altered gravitational fields. Genes of interest were selected from a larger group, the expression of which was altered under hypergravity (microarray study; Martzivanou and Hampp, *Physiologia Plantarum* 118, 221-231, 2003). Transcriptional changes of these genes were studied within a time frame of up to ten minutes of exposure to microgravity in a sounding rocket experiment (TEXUS), to clinorotation (random positioning machine), and to hypergravity (8 g). We could identify a set of six genes (mainly components of signalling chains) with increased transcript levels after about six minutes of exposure to microgravity. As clinorotation and hyper-g treatment did not alter the respective transcript amounts, we assume that the identified genes could be involved in a microgravity-related response.

P0313. Transcriptional regulation by CsARF2 and CslAA1 for the gravity-regulated morphogenesis of cucumber seedlings

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Cucumber (*Cucumis sativus* L.) seedlings develop a peg on the concave side of the gravitropically bending transition zone between the hypocotyl and the root. Peg initiation occurs due to auxin accumulated in the concave side of the transition zone through graviresponse. Auxin and mRNA of auxin-responsible

genes accumulated more abundantly in the lower side of the transition zone in a horizontal position, compared to those in the upper side. Also, mRNAs of *CsARF2* and *CslAA1* that encode transcriptional regulators of auxin-responsible genes, concomitantly accumulated in the transition zone at the stage of peg initiation. Tobacco BY-2 protoplast transient assay showed that *CsARF2* activated the transcription of auxin-responsible promoter, DR5 and IR3, in the presence of auxin. These results suggest that *CsARF2* is a transcriptional activator for the auxin response required for peg formation. We will discuss the role of *CslAA1* in the transcriptional activation of *CsARF2*.

P0314. Functioning of rye seedlings adenilatcyclase system under the microgravity influence.

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Role of adenilatcyclase system (AC) in resistance formation to various stress factors is well known. We have studied cAMP content, phosphodiesterase (PDE) characteristics in rye seedlings under the microgravity influence caused by klinostating (4 turns per minute).

3-day seedlings exposition to microgravity have decreased vegetative and root mass, changed their spatial orientation. Short-term klinostating have increased cAMP level. Retrieving seedlings to steady-state conditions have decreased this index to control values. At this, PDE cAMP activity has decreased almost by three times resulting in increase of cAMP level and enzyme sensitivity to Ca²⁺ and Ca²⁺-calmodulin.

Enzyme was found to be sensitive to phytohormones - cytokinins and ABA. It was inhibited by zeatin and stimulated by ABA.

Role of AC both in the mechanism of ABA action and the importance of phytohormone in regulation of AC cascade reactions was indicated. Initial increase of cAMP level acts as signal for the adaptation processes initiation and is reversible. Change of its characteristics cause increase of cAMP level and initiation of adaptation to stress action of microgravity.

P0315. MADS about moss

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Classic *MIKC*-type (*MIKC*^c) MADS-box genes are indispensable elements in genetic programming of pattern formation, including segmental organisation of angiosperm flowers, in seed plants. Since little is known about functions of *MIKC*^c genes in non-seed plants, functional analysis of moss *MIKC*^c homologues was performed with the genetically amenable species, *Physcomitrella patens*.

Expression of moss homologues was knocked down by antisense RNA or abolished by single and multiple targeted gene knockouts. Knocked down (antisense) transformants displayed a multifaceted mutant phenotype comprising abnormal leaf morphogenesis, delayed gametangia formation and abnormal sporophyte development. Most knocked out transformants were phenotypically normal. A single exception possessed the complex mutant phenotype that characterised the antisense strains.

MIKC^c genes play significant roles in morphogenetic programming of the moss. Functional redundancy characterises some members of the gene group. Our findings provide clues concerning the ancestral roles of at least some *MIKC*^c genes that may be represented in the genomes of diverse extant plant taxa.

P0316. Functional analysis of class 2 KNOX gene in *Physcomitrella patens*

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KNOX homeobox genes have an important role in initiation and maintenance of shoot apical meristem and they are divided into two subfamilies, class 1 and 2. The functions of class 1 genes have been characterized by mutant analyses and overexpression experiments, while the functions of class 2 genes are still unknown, because loss-of-function mutants have not been reported and any phenotype has not been reported in its over-producing transformants.

Physcomitrella patens Bruch & Schimp subsp. *patens* is a suitable model plant to analyze genes with unknown function, since gene

targeting techniques have been established in this moss. We are investigating the function of a KNOX class 2 gene, *MKN1* (Champagne and Ashton 2001). The expression pattern of *MKN1* was analyzed by RT-PCR. *MKN1* mRNA was expressed in gametophores with gametangia and embryonic sporophytes, but not in protonemata nor gametophores without gametangia. Detailed expression pattern is unraveled by transformants, in which *uidA* (GUS gene) is inserted at the end of *MKN1* coding sequence. Phenotypic analyses of *MKN1* disruptants are presented.

P0317. Plant homologs of bacterial peptidoglycan biosynthesis genes in moss

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It is now widely agreed that all plastids in red and green algae, land plants and glaucocystophytes originated from a single cyanobacterial ancestor. Therefore, the evolution from endocytobiont into a wall-less, photosynthetic organelle involved a loss of the cyanobacterial cell wall. The peptidoglycan synthesis pathway is a major target for antibiotics. In the moss *Physcomitrella patens*, treatments with β -lactam antibiotics including ampicillin and penicillin, D-cycloserine and fosfomycin, which inhibit peptidoglycan synthesis at different steps, resulted in giant chloroplasts. Moreover, ampicillin inhibited plastid division in the liverwort *Marchantia polymorpha* and Pteridophyta *Selaginella nipponica*. We isolated 9 genes that were related to peptidoglycan synthesis from *P. patens*. Seven of them were predicted to have plastid-targeting sequences by the TargetP program. These findings suggest that a relic of the bacterial peptidoglycan synthesis pathway is retained in moss plastids.

P0318. Role of the PpPOK protein, an homolog of the yeast Vps52p protein involved in vesicle trafficking, in the protonema growth of *Physcomitrella patens*.

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The Vps52p protein of *Saccharomyces cerevisiae*, has been shown to be essential in retrograde vesicular transport to the late Golgi in a complex with other proteins, Vps53p, Vps54p, and Vps51p (Conibear et al., 2003). Vps52p is highly conserved from lower to higher eukaryotes. The Arabidopsis homolog of Vps52p, named POK (poky pollen tube), is essential for normal pollen tube growth and the mutation is lethal when homozygous (Lobstein et al., 2004). These phenotypes and the POK gene expression in most plant tissues suggest a general role for POK in polar growth processes. In order to precise the function of the Vps52p homolog in plants, we extended its study to mosses, which protonema represent, as pollen tube, excellent unicellular tip-growing cells experimental systems. Moreover, the high levels of homologous recombination in *Physcomitrella* allow specific genes to be targeted and their functions thereby studied. We have identified the *Physcomitrella* POK homolog, designed PpPOK. The PpPOK protein shows 94% identity with the Arabidopsis POK protein. Knockout transformants for PpPOK have been generated and their phenotypical characterisation is in progress.

P0319. What makes leafy gametophytes turning

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Chiral aspects of phyllotaxis in leafy gametophytes of true mosses have been much less studied than in sporophytic shoots of other plants. The direction of leaf initiation in the gametophyte i.e. the configuration of phyllotaxis, reflects the order of divisions of its single apical cell because leaves are clonally related to merophytes. When divisions are parallel to the sides of the base of the apical cell, as it is in *Fontinalis antipyretica*, every 4-th merophyte rises exactly in the same circumferential position of a shoot. As a result tristichous phyllotactic pattern develops with three vertical ortostichies. When divisions are oblique, as in *Polytrichum commune*, every 4-th merophyte is circumferentially displaced - presumed ortostichies become bias. As expected, both chiral configurations occur in the two species. However, branching system of *Fontinalis* shows a peculiar, hard to explain dependence of the configuration of the lateral stems on that of the supporting ones. In *Polytrichum* the ortostichy deviation from the vertical

course is concordant with the direction of leaf initiation although, theoretically, the opposite relationship is possible.

P0320. Spore ultrastructure in five Canarian species of pleurocarpous mosses.

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Ultrastructural researches in moss spores have yielded interesting results, although it is still difficult to assess their systematic or adaptative meaning.

We selected five species from the Canarian laurisilva forest: *Leucodon canariensis* (Leucodontaceae); *Leptodon longisetus*, *Neckera cephalonica*, and *Neckera intermedia* (Neckeraceae), and *Isoetecium myosuroides* (Brachytheciaceae). They all coexist in this very special habitat and share similar ecological requirements. Mature spores were studied using SEM and TEM. Spore size frequencies were also analyzed. This research is carried out for the first time in these families.

All five species are distinct according to spore characters. The spore of *Leucodon canariensis* stands apart due to its bigger size, pluricellularity, and presence of an aperture. A bimodal distribution of the spore size indicates anisospory in both *L. canariensis* and *L. longisetum*. In the latter species, we discuss the spore features supporting its placement into a new family, Leptodontaceae, as recently suggested.

P0321. Changes in cortical microtubule organization in scale cells of *Narcissus tazetta* entering dormancy stage

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Dormancy induction and completion can not be determined visually in geophytes and is defined as the time where no visible changes of the bulbs are observed. The annual life cycle of *Narcissus tazetta* represents a classic example of the "Mediterranean" type of bulb where dormancy initiates at spring, last all summer and the mild drop of temperatures to 8-17 C in the winter fulfill the requirements for flowering. In this research we use molecular cell biology techniques to characterize intra-cellular changes delineating different stages of dormancy in cells of *Narcissus tazetta* bulbs. We found that amyloplasts increase in size gradually from November (10-15  m) till May (25  m in diameter). During this time the organization of cortical microtubules changes from parallel arrays to a net encircling the big amyloplasts. This unique organization changes back into parallel arrays towards the months of June-July. We continue to characterize this phenomenon, both *in-vivo* and *in-vitro* in order to verify a possible cross talk between microtubules and amyloplasts membranes in cells of scales of *Narcissus tazetta*.

P0322. Phospholipase D activation is involved in the macro-tubule-dependent protoplast volume regulatory mechanism in plasmolyzed root-tip cells of *Triticum turgidum*.

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The involvement of phospholipase D (PLD)-produced phosphatidic acid (PA) in the macro-tubule-dependent protoplast volume regulation [*Plant Cell Physiol.* (2002) 43: 911] was studied in plasmolyzed wheat root cells. In these cells, the effects of butanol-1, NAE and PA were assessed on the hyperosmotic response and the macro-tubule organization. Butanol-1 and NAE, which inhibit PA production, induce a marked reduction in the plasmolyzed protoplast volume and inhibit the hyperosmotically-induced macro-tubule formation. By contrast, PA potentiated the hyperosmotic response of root cells while inducing supernumerary macro-tubules. Results from western blot analysis suggest that the PLD/PA system acts upstream of a MAPK pathway as treatment with the above inhibitors modulated the accumulation of a 46 kDa phosphorylated p38 MAPK-like protein in plasmolyzed roots. This protein was previously shown to interfere with macro-tubule formation [*FEBS Lett.* (2004) 573: 168].

P0323. Effect of red and blue light on the orientation of cortical microtubules in epidermis of sunflower hypocotyl

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The effects of red and blue light and auxin on the orientation of cortical microtubules (cMTs) under the outer epidermal walls of sunflower hypocotyls were investigated. The strips of epidermis were isolated, then incubated for 12 hours in Murashige & Shooq medium with or without auxin and irradiated with blue or red light for 1 hour.

The orientation of cMTs was transverse or oblique with respect to the cell axis, independently of the auxin's presence or irradiation. Many histograms showing frequencies of parallel cMTs versus orientation angle exhibited asymmetrical distribution with prevailing of right-handed cMT arrays. The irradiation with red light caused increase in the amount of asymmetrical histograms in which right-handed arrays of cMTs were dominated. Taking into consideration that the autonomous reorientation of cMTs is rotational (Hejnowicz, Protoplasma 2005, in press) we propose that the red light influences chirality of the cMTs by modulating the velocity of autonomous reorientations of the cMTs in the rotational cycle.

P0324. Polyglutamylation effects on the spatial structure and molecular dynamics of plant tubulin

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Polyglutamylation has been detected as post-translational modification of animal α - and β -tubulins. This modification is typical for stable microtubule populations, and can be involved in microtubule-MAP interaction. Polyglutamate side chains of various lengths can be attached to multiple neighboring glutamate residues on C-terminus of tubulin molecules. Using specific GT335 antibody we found polyglutamylation of α - and β -tubulins in *Daucus carota*. To study influence of this modification on tubulin molecular dynamics, the 3-D models of *D. carota* polyglutamylated tubulins were reconstructed. (Glu)_n were attached to Glu445 of α - and to Glu432 of β -tubulin. Molecular dynamics was calculated during 1 nsec using GROMACS software. It was found, that addition of (Glu)₂ and (Glu)₄ reduced levels of molecular oscillations of β -tubulin, but does not influence on α -tubulin dynamics. Addition of (Glu)₆ resulted in increasing of β -tubulin molecular dynamics, but does not modify α -tubulin behavior. Thus, reduction of tubulin molecule oscillation by adding glutamate residues results, in fact, in increasing of its stability was confirmed by *in silico* methods.

P0325. Mechanisms of plant resistance to dinitroanilines and phosphoroamidates based on β -tubulin mutations

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Early we produced *Nicotiana plumbaginifolia* β -tubulin mutants resistant to amiprofosmethyl (APM), a phosphoroamidate herbicide (PAH), with cross-resistance to trifluralin, dinitroaniline herbicide (DNH). Altered tubulin formed APM-resistant microtubules after transfer of this trait to another species by somatic hybridization. β -Tubulin genes from control and mutant lines were sequenced, and replacement of Ser248 to Pro in one of beta-tubulin gene from mutant was established. Comparative analysis of 3-D structures of sensitive and resistant β -tubulins identified location of this residue in zone of tubulin intradimeric interaction. Ser248 as well as Lys252 and Lys350 participates in cavity formation, which can be identified as PAH/DNH interactive site, and in creation of the microenvironment for ligand-tubulin interaction. Substitution of Ser248 to Pro results in the blocking of interactive cavity. These results coincide with our data on reconstruction of spatial structure of *Chlamydomonas* mutant β -tubulin, obtained by Schibler and Huang (1991), where crucial role of missense mutation of Lys350 in colchicine- and PAH/DNH-resistance was found.

P0326. Expression of the *Nicotiana tabacum* pollen-specific gene NTP303 is required for pollen tube growth and fertilization.

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We isolated a pollen-specific gene from tobacco, NTP303 with four other family members. NTP303 transcripts accumulate in pollen, but the protein is only found at germination and during pollen tube growth. Presently, we analyzed the effect of NTP303 gene silencing on pollen function. The kanamycin resistance trait linked to the NTP303-silencing gene was not transmitted through the male gametophyte. Since we could not find a readily distinguishable phenotype in pollen from the hemizygous plants, we produced double-haploid plants homozygous for the NTP303 anti-sense gene. In pollen from these plants, transcript levels of all NTP303 family members were strongly reduced. Pollen and pollen tubes grown *in vitro* appeared completely normal. However, the pollen tubes *in planta* showed lower growth rates and their growth became arrested in the style, so that fertilization failed. These data indicate that NTP303 and its family members are essential for pollen tube growth *in planta*. Together with the localization of the NTP303 protein in the wall and at the plasma membrane of the pollen tubes, the present results suggest a function in cross talk between pollen tube and pistil.

P0327. A role for the exocyst complex during plant development.

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The exocyst is a eight subunits protein complex conserved among eukaryotes. Characterised in yeast and mammals, it was shown to participate in the spatial regulation of exocytosis. Acting as a tether, the exocyst physically links secretory vesicles with localised plasma membrane subdomains before fusion. Homologues of each exocyst subunit were identified in the Arabidopsis thaliana genome [1]. And a putative exocyst-like complex involved in vesicles tethering was recently described in Arabidopsis [2].

In this study, we describe Arabidopsis RNAi lines in which we were able to suppress the expression of Sec3 and Sec5 exocyst subunits. A mutant phenotype was observed in early plant development under stress conditions. We used antisense oligonucleotides to analyse the exocyst function in tobacco pollen development. The exocyst appeared to be involved in pollen tube elongation. These results indicate the exocyst complex may have a role to play in different processes of plant development.

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[1] Elias et al. 2003 Cell Biol Int 27:199

[2] Segui-Simarro et al. 2004 Plant Cell 16: 836

P0328. Localization of the exocyst complex in plant cells

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Highly conserved protein complex called the exocyst is thought to participate in tethering of secretory vesicles to the plasma membrane. The exocyst has been described in mammalian and yeast cells. However analysis of plant genome sequences revealed the presence of homologues to all eight exocyst subunits (Elias et al. 2003), indicating that the exocyst complex is present also in the plant cell. Most of exocyst subunits are encoded by more than one gene.

Our analysis of public expression data based on the Affymetrix DNA chip revealed that some genes are tissue specific. We have focused on highly expressed or unique genes and constructed several GFP fusions. Using the method of transient expression in tobacco leaves and an immunolabeling of pollen tubes, we observed predominantly cortical and perinuclear localization of these fusion proteins. The localization to cell invaginations and tips of pollen tubes suggests the role of the exocyst in the spatially localized exocytotic pathway.

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Elias et al.: The exocyst complex in plants. Cell Biol Int. 2003; 27(3):199-201.

P0329. Crosstalk between different signaling pathways in pollen tube growth and reorientation

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Pollen tube growth and re-orientation are dynamic cellular processes important for a successful fertilization. Understanding the guidance mechanisms involved has been our main research focus and important insights into the biochemical and physiological aspects of these events have been reported. Nevertheless, much is still unknown. Here we describe recent findings of our laboratory on the regulation of endo/exocytosis as a motor for pollen tube growth and how the secretory machinery depends on the modulation of intracellular Ca²⁺ levels. Ion pumps and channels, Ca²⁺-dependent protein kinases, phosphoinositides and phospholipids, all seem to play an active role in this process. To study the cross talk between these different signalling pathways is thus the thrive of our work. Here we show new data on the intracellular perception of guidance cues and how this affects endo-exocytosis. The results support a model where directioning is dictated by the combined action of several molecules and not by a simple cascade of events.

P0330. Phospholipase D signaling cascade regulates polar cell expansion

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Phospholipase D (PLD) is an important regulating enzyme involved in many processes in animal cells while its role in plants remains largely unknown. PLD cleaves phospholipids producing phosphatidic acid (PA), an emerging second messenger in plant cells.

We studied PLD roles in regulation of vesicle trafficking within plant cell employing tobacco pollen tubes as a model system. Application of 1-butanol, the inhibitor of PLD signaling, rapidly affected pollen tube cytoarchitecture and vesicle dynamics as visualized by video-enhanced microscopy. Conversely, exogenous PA was able to stimulate pollen tube growth and trafficking dynamics.

To characterize distinct PLD isoforms, we used gene specific knock-down mediated by antisense oligos. The suppression of selected PLDs lead to lower pollen tube growth rates, confirming the importance of PLD signaling for polar growth and raising the question of downstream targets of PA. We suggest that different PLDs regulate cytoskeletal dynamics and production of reactive oxygen species generated by NADPH oxidase.

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P0331. Disturbance of endomembrane trafficking by Brefeldin A and Calyculin A reorganizes the actin cytoskeleton of *Lilium* pollen tubes

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We investigated the effect of Brefeldin A (BFA) on the actin cytoskeleton of pollen tubes of *Lilium longiflorum*. The formation of a subapical BFA-induced membrane aggregation (BIA) was associated with the formation of an actin basket from which filaments extended towards the tip. Their orientation correlated with the trajectories of FM-stained membrane material suggesting that the BIA-associated actin filaments are used as tracks for retrograde transport. These tracks (actin filaments) were either stationary or glided towards the BIA together with the attached membranes. Treatment with Cytochalasin or Latrunculin caused arrest of membrane trafficking, dissipation of the BIA and the actin basket. Our observations suggest that BFA causes ectopic activation of actin nucleating proteins at the BIA resulting in retrograde movement of membranes not only along but also together with actin filaments. We show further, that subapical

membrane aggregations and actin baskets supporting retrograde membrane flow can also be induced by Calyculin A, indicating that dephosphorylation by type 2 protein phosphatases is required for proper formation of membrane coats.

P0332. Phospholipid signaling in pollen tubes of *Nicotiana tabacum*

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Pollen tube growth is essential for plant fertilization and reproductive success. Pollen tubes expand by extremely polarized and rapid growth. Multiple signal transduction cascades are controlling fast organelle movements, targeted secretion and a tip-focused Ca²⁺ gradient, which are required for pollen tube growth. Phosphoinositide-specific phospholipases C are enzymes that hydrolyze phosphatidyl-inositol-4,5-bisphosphate (PIP₂) and play key roles in diverse signal transduction processes in all organisms. Five PI-PLC isoforms have been identified in mammalian cells. The enzymatic activities of each of these types of PI-PLC isoforms are regulated by distinct mechanisms. The functions and regulation of plant PI-PLCs are not well understood to date. A number of plant PI-PLCs have been cloned so far, e.g. from Arabidopsis, tomato, pea and soybean. They all are most similar to the mammalian class of PI-PLCs delta and/or zeta. We identified two pollen-specific PI-PLCs from *Nicotiana tabacum*. The Ca²⁺-dependent enzymatic activities of wild type and various mutant forms of NtPLC4 have been determined and in vivo functional analysis has shown that PI-PLCs are key regulators of polar pollen tube growth.

P0333. Oscillations in pH anticipate growth in lily pollen tubes; a role for actin

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Lily pollen tubes exhibit oscillations in their rate of growth (100-600 nm/sec), which are accompanied by oscillations in calcium and protons in the apical domain. We have compared these oscillatory events (e.g., growth vs. protons) using cross-correlation analysis to determine if a process precedes or follows growth. Our results show that increases in alkalinity anticipate growth by 100 degrees, suggesting that pH is a possible regulator of growth. Exploring the relationship between pH and actin polymerization, we show the following: 1) The oscillating alkaline band colocalizes with the cortical actin fringe. 2) ADF, an actin binding protein, which stains the actin fringe, is pH sensitive; at elevated pH it enhances the turnover of actin. 3) The culture of pollen tubes in sodium acetate causes acidification of the apex, and a rapid decay of the actin fringe. Since actin polymerization is essential for pollen tube growth we suggest that proton pumps extrude protons creating an intracellular alkaline band. The elevated pH activates ADF, which then fragments F-actin. The actin fragments stimulate polymerization and facilitate growth of the pollen tube.

P0334. Molecular identification of 14-3-3 isoforms of lily pollen and their possible role in activation of the plasma membrane H⁺ ATPase

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Members of the highly conserved 14-3-3 protein family were detected in virtually all eukaryotic cells. They are involved in essential cellular processes including plant defence and stress responses, plant development, signal transduction, and modulation of enzyme activities. The role of 14-3-3 proteins depends on their intracellular localisation and on the interaction of specific 14-3-3 isoforms with phosphorylated target proteins. In lily pollen grains and tubes 14-3-3 proteins are associated with all organelle membranes and show a fusicoccin-dependent binding to plasma membranes activating the PM H⁺ ATPase (Pertl et al. 2001, 2005). In order to investigate the isoform specificity of this interaction, cDNA libraries obtained from pollen grains and tubes, respectively, were screened with a degenerated digoxigenin-labelled probe. So far, 4 different 14-3-3 isoforms were identified and partially sequenced. The interaction of the various isoforms with the PM H⁺ ATPase will be tested by immunodetection in an overlay blot using a plasma membrane-enriched fraction and native as well as recombinant 14-3-3 proteins obtained from lily pollen.

P0335. Molecular identification of a putative K⁺ inward channel in lily pollen

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An influx of potassium ions is necessary for the germination of pollen grains on compatible stigmas as well as in *in-vitro* pollen cultures. The plasma membrane H⁺ ATPase hyperpolarizes the plasma membrane more negative than the K⁺ equilibrium potential and thus energises the K⁺ influx mediated by K⁺ inward channels. During germination of pollen grains and growth of pollen tubes different types of K⁺ inward currents were detected and characterised in *Lilium longiflorum* pollen grain, pollen tube, and pollen tube tip protoplasts using the whole-cell configuration of the patch-clamp technique. The identification of the responsible K⁺ channel genes was performed by RT-PCR using K⁺ channel-specific primer, by screening cDNA libraries obtained from lily pollen grain and tubes with a digoxigenin-labelled probe, and by SMART-PACE-PCRs, respectively. In this study we present the first results on K⁺ inward channel sequences expressed in lily pollen grains and pollen tubes.

P0336. Ammonium: A major nitrogen source for plant nutrition in ectomycorrhizal symbiosis?

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In ectomycorrhizal symbiosis, plant fine roots are isolated from soil by a fungal sheath, making plant nutrition dependent on the fungal partner.

To understand plant nitrogen nutrition in symbiosis, a total of 7 putative high affinity ammonium importer genes were identified within the *Populus trichocarpa* genome. One gene (PoptrAMT1.1) was expressed only in roots, and 4 genes were detectable to a different extent in all poplar organs.

Ectomycorrhiza formation resulted in a strongly increased (5- to 9-fold) transcript level for those 3 genes (including PoptrAMT1.1), that are most prominent in fine roots.

The transport properties of the root-specific transporter were investigated by heterologous expression in a yeast mutant, revealing a K_m value of about 52 μM, and thus indicating the protein as high affinity ammonium uptake system.

The enhanced expression of 3 poplar genes encoding putative ammonium uptake systems in mycorrhizas indicates a strongly increased ammonium uptake capacity of tree roots in symbiosis. This result is a first hint that ammonium could be a major nitrogen source that is delivered from the fungus to the plant in ectomycorrhizas.

P0337. Tree roots and underground infrastructure - a problematic relationship

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Tree roots intruding sewer pipes and drains lead to blockages. The real causes for the roots to find and enter the pipes are still unknown. In the past, leaking joints were supposed to cause these damages. Analysing excavated joints we could reject this theory. Furthermore, our investigations have shown that neither the water inside the pipes nor the nutrients carried with the water represent the leading causes for the roots to penetrate the pipe-joints. Instead we could set up the density-trap-model. Empty space in the joint, outside the sealing ring, leads to a density gradient which is followed by the growing root. Mechanical measurements of forces exerted by growing roots showed forces from 5 bar (*Pisum sativum*) up to 12 bar (*Quercus robur*). This indicates clearly, that resistance to inside overpressure of 0,5 bar as defined by industrial norms does not guarantee root-proof joints.

P0338. Shoot and root length are controlled by separate genes in barley mutants

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Nine barley mutants with seminal roots significantly shorter than roots of parent varieties have been identified in the collection of dwarf and semi-dwarf forms of the Department of Genetics, University of Silesia. Genetic analysis performed at seedling and

spike emergence/maturity stage indicated that a single recessive gene was responsible for root and shoot shortening in each of analysed mutants. The presence of recombinants with short root, long shoot (or opposite) in F₂ generation of the crosses 'mutant x parent' proved that root and shoot length were controlled by independent loci. Reciprocal crosses between four mutants revealed that they were non allelic. In two mutants (225DV from cv.'Diva'; 035AR from cv. 'Aramir'), the linkage between genes responsible for root and shoot length was revealed. Molecular mapping with AFLP and SSR markers indicated that genes responsible for root and shoot length in mutant 225DV were located in the centromere region of chromosome 5H. Genes controlling root and shoot phenotype of mutant 035AR were localized in the distal part of chromosome 7H.

P0339. Structural and functional traits of scion-rootstock interactions in cherry tree (*Prunus avium* L.) under drip-irrigation

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Anatomical and functional traits of cvs Burlat and Summit grafted on Gisela 5 (dwarfing rootstock) and on *Prunus avium* (invigorating) were studied during 2003. Leaf gas exchange, photosynthetic pigments, carbohydrates and total phenols concentrations and anatomical parameters (by Scanning Electron Microscopy) were measured on each scion-rootstock combination. Higher values of net CO₂ assimilation rate, stomatal conductance, transpiration rate and internal CO₂ concentration were always observed in trees grafted on *Prunus avium*. In opposition, higher concentrations of total chlorophyll and carotenoids were presented by trees on Gisela 5. Higher starch and total phenols concentrations were determined in Burlat. In addition, Burlat presented higher stomatal density, particularly when grafted on the dwarfing Gisela 5. Summit on Gisela 5 exhibited a thicker mesophyll tissue (palisade and spongy parenchyma) and a thicker lower epidermis, traduced in a higher total lamina thickness. The relationship between physiological and morphological parameters is discussed by scion-rootstock combination.

P0340. Physiological functions of ammonium transporters of the AMT family in plants

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Proteins of the AMT family are believed to represent the major transporter class for the membrane transport of ammonium in plants. So far, correlations between transcript levels and ammonium influx have been used to attribute putative physiological roles to AtAMTs, but the contribution of individual AMT homologs to ammonium uptake remained unclear.

To investigate the physiological role of AtAMTs, our group isolated a number of Arabidopsis lines carrying transposon or T-DNA insertions in *AtAMT1;1*, *AtAMT1;2*, and *AtAMT1;3*. These lines were tested for influx of ¹⁵N-labelled ammonium into roots and subjected to a phenotypical analysis under different growth conditions. Furthermore, tissue-specific gene expression and membrane localization were determined besides the posttranscriptional regulation of AMT-dependent ammonium transport using specific antibodies and overexpression lines. A summary of the actual results will be given and a model will be presented for the proposed physiological functions of individual AMTs in roots.

P0341. A new approach to analyze morphological heterogeneity within grass root systems

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Uniform activity among all roots is a common assumption in most studies of root physiology. It is almost certainly grossly inaccurate, but little information exists with which to formulate a description of the heterogeneity within root systems. Here, a new approach, based on the segmental morphology of grasses, was used to categorise root ages and analyze the effect of defoliation on the relationships between root age and morphology in *Lolium perenne*.

Defoliation strongly reduced total root length. This was mainly due to a drastic effect on root elongation at phytomers which were branching at the time of defoliation. Defoliation also reduced total root mass. However, specific root length (SRL, mm mg⁻¹) was only slightly affected. This was due to two contrasting responses: on one hand, new root tissue had higher SRL, and on the other, this was counter-balanced by concomitant decreases in branching, and therefore in SRL (branches are thinner than main axes and have higher SRL). These results emphasise that responses of whole-root system should be carefully interpreted for they are integrated by quite contrasting responses of mature compared to actively growing roots.

P0342. How similar are the tuberous roots between *Pterocactus* and *Peniocereus*?

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We document structural modifications of tuberous roots in *Pterocactus* (7 species) and *Peniocereus* (18 species) that belong to Opuntioideae and Cactoideae subfamilies to support the hypothesis that storage roots arise independently in Cactaceae. The species of *Peniocereus* are distinctive by their napiform large tuberous root or fasciculated roots, but no adventitious storage roots or subterranean stems were observed, while in the *Pterocactus* species, there is a large tuberous root that acquires an irregular shape depending on plant age, but additional storage roots having a caulinar origin and subterranean stems were common. Among the distinctive root anatomical differences between *Pterocactus* and *Peniocereus* species are: widening of parenchyma cells in the root central core, secondary xylem with wide band tracheids and druses or absent in *Peniocereus*, secondary phloem near the periderm in *Pterocactus* or separated by an extensive parenchymatous region, lack of sclerenchyma in tuberous roots or present in *Peniocereus*. Anatomical differences suggest that tuberous roots in Cactaceae have an independent origin.

P0343. Characteristics of Root Structure in Tea Plants

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Tea plants (*Camellia sinensis* L.) are evergreen and long-living shrubs. Because the knowledge of tea root anatomy is quite limited, we have studied the structure of seminal and adventitious roots under different conditions. Seeds of tea cultivar Yabukita were grown in perlite in greenhouse. Cuttings of the same cultivar were planted in the field, hydroponic-cultured in the greenhouse and grown in the mist chamber. Roots of those plants were stained with berberine - toluidine blue and fluorol yellow in order to visualize Casparian bands and suberin lamellae, respectively. Casparian bands were developed both in exodermis and endodermis close to the apex. Endodermal Casparian bands were formed at 0.1, 0.2, 0.5 cm from the root tip in soil, mist and hydroponic culture respectively. Exodermal Casparian bands were developed at 0.1 cm in soil, 0.1 cm in mist culture and 0.2 cm in hydroponic culture. Such development of functional exodermis sooner than endodermis in tea roots is quite exceptional. This early development of apoplastic barrier close to the root tip is suggested to play an important role in regulation of radial transport of water and minerals in tea roots.

P0344. Competitive ability of the root systems of beech and spruce: a space-related cost-benefit analysis

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In a 60-year-old mixed forest of *Fagus sylvatica* and *Picea abies* structure and function of the roots of both species have been analysed and compared. To determine their competitive ability, the space occupied per unit of root biomass, the amount of water extracted by the roots per unit of occupied space, and the space-related 'running costs' of respired carbon were quantified. Although the coarse-root systems as evaluated through ground-penetrating radar were similar in both species regarding rooting depth and total biomass, substantial differences existed in physiological traits, when dividing the fine roots into functional classes. The latter were

characterised by lignification and mycorrhization rather than root diameter. By identifying types of ectomycorrhizae which distinctly differ by the radii explored around the root tips, the effectively occupied space could be approximated. The exceptionally dry summer of 2003 was used as a test scenario which showed beech roots to be less affected by prolonged water shortage and to have competitive advantages over spruce.

P0345. Fast alterations in root cell patterning precede the changes in root architecture induced by aluminium stress.

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Inhibition of root cell elongation has been made responsible for the fast Al-induced decreased of root growth. However, inhibition of cell elongation alone may not explain the subsequent alterations in the entire root architecture of Al stressed plants. Here the influence of short-term Al exposure on cell division in roots of maize was investigated using fluorescence confocal microscopy detection of immunolabeled S-phase nuclei. A 5 minutes Al exposure was enough to inhibit cell division in the meristem (250-800 µm from tip). After 10 or 30 min with Al only a few S-phase nuclei were found in the cortical initials. In contrast, cell division was stimulated in the distal elongation zone (2.5 to 3.1 mm). After 3 h the protrusion of lateral root was observed in this zone. Similar changes were induced by a local supply of NPA. This suggests that inhibition of auxin transport plays a role in the fast Al-induced alteration of root cell patterning that underlies the drastic change in root architecture. *Acknowledgement: Supported by DGICYT (BFU2004-02237CO₂-01) and Pla Recerca Catalunya 2001 (SGR00200).*

P0346. Comparative study of cellular structures implicated in gravisensing in the statocytes of primary and lateral roots of *Vigna angularis*

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The lateral roots already had the statocytes with small amyloplasts sedimented in their lower end immediately after they emerged from a primary root. Nevertheless, they showed plagiogravitropism in an early growth stage. Both in primary and lateral roots, all the statocytes had cortical microtubules which showed various arrays and actin filaments appeared as cables or bundles in the root-cap peripheral cells, whereas they did not clearly seen in mature statocytes with sedimented amyloplasts. Noteworthy, the endoplasmic reticulum (ER) developed much better in primary roots than in lateral roots. The tubular ER cisternae were suggested to form a large complex in the lower part of the statocytes of primary roots and the amyloplasts appeared to settle on the upper surface of this complex. Whereas, only a few cisternae were seen in the statocytes of lateral roots. Thus, it is suggested that the differential developmental level of the amyloplasts-ER complex system strongly correlates with differential gravitropism of the two types of roots.

P0347. Root system morphogenesis and comparative anatomy of Fabaceae species in relation to the environment

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The anatomy, dimension, and distribution of the root system of **Fabaceae** species seem to be related to the exploration and efficacy of the exploitation of resources. These factors vary under different environmental conditions and according to the different genotypes. The morphogenesis and anatomy of the root system of the species indigenous to Argentina of *Adesmia*, *Aeschynomene*, *Chamaecrista*, *Coursetia*, *Desmanthus*, *Hoffmannseggia*, *Indigofera*, *Lathyrus*, *Poiretia*, and *Rhynchosia* were studied particularly in relation to the environment. The development of the root system was observed through glass-sided containers. Serial transversal cuts of the roots were performed. The histological traits that determine the seven structural root models found, are the following: xylematic/phloematic relationship, presence and dimension of radii, quantity of cambia, cells produced by the cambium, presence and distribution of reserve substances. Adaptation strategies were also inferred.

P0348. Endocytosis and vesicle trafficking in root hairs

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Tip growth of root hairs depends on regulated vesicular trafficking; vesicles supply new cell wall and membrane components to the tip by exocytosis and they mediate the recycling of the material from the tip by endocytosis. So far, however, little is known about the dynamics of endocytosis in living root hairs.

We analyzed endocytosis and the behaviour of vesicles in living root hairs of *Arabidopsis* and of *Triticum* using fluorescent endocytosis marker dyes FM1-43 and FM4-64. We showed active endocytosis at the tip and further trafficking of the endocytosed membranes through highly dynamic early endosomes, late endosomal compartments and the tonoplast. We found that rapid endocytosis of the plasma membrane needed the actin cytoskeleton, and that vesicle trafficking but not rapid endocytosis was interrupted by Brefeldin A. Moreover, complexation of structural sterols in the plasma membrane of root hairs altered the physiological properties in the tip and compromised regulated endocytosis.

Results describe specific endomembrane compartments of the endocytotic pathway during the tip growth of root hairs.

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P0349. Measuring aquaporin activity in a protoplast swell assay: contribution of the non-osmotic volume

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Due to the growing interest in the contribution of aquaporins (AQP) to water transport in plant cells, protoplast swell assays are performed routinely to test for the physiological functionality of AQPs. Usually, the water permeability coefficient (P_{os}) is determined according to a method used for *Xenopus* oocytes, in which the water uptake is monitored by a time-dependent volume change upon a hypoosmotic shock. Although it is commonly known that a certain fraction of the cell content does not participate in the osmotic process (fractional non-osmotic volume β), it was neglected in osmotic swelling experiments on plant cells. In this context, we developed an analysis method to determine the non-osmotic volume from the time-dependent volume changes of single-cell measurements and tested this method on protoplasts of different origins (lily pollen and mesophyll tissue from *N. tabacum* and *A. thaliana*). Average β -values around 30% were obtained. We compared these results with β -values calculated using the "classical" Boyle-van't Hoff plot and analysed the impact of β on calculating P_{os} .

P0350. Reactions of growing root hairs to hypertonic media

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Root hairs are a plant's contact site with the environment and the first to sense changes in the soil. In order to test their reactions to osmotic stress, we observed root hairs of 3 day old seedlings of *Triticum aestivum*. Plants were exposed to iso- and hypertonic solutions of glucose, mannitol and polyethyleneglycol (PEG) in concentrations of 100 - 500 mOsm.

Roots and root hairs adapted to hypertonic solutions of glucose by taking up considerable amounts which increased their osmotic value. Contrary to established opinion, also mannitol was taken up, thus allowing for initiation and growth of new root hairs in hypertonic solutions. In PEG, however, new root hairs were formed only up to isotonic concentration, it was not taken in. During plasmolysis, the polar organisation of the cytoplasm persisted and cell wall formation continued.

Endocytosis of the plasma membrane was tested with membrane-impermeable fluorescent dyes like FM1-43. We compared membrane recycling during tip growth with the behaviour of the plasma membrane during plasmolysis

P0351. Intrusive growth of the cambial initials between periclinal walls and its influence on the arrangement of wood cells: a critical evaluation

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The mechanism of change in the orientation of cells in dicotyledonous wood, like fibres, parenchyma and vessel elements, is poorly understood. Observations suggest that the degree of inclination and the rate of changes in orientation of the axial wood elements, creating curly waves or interlocked grain in the wood, depend on the unidirectional intrusive growth of the fusiform cambial initials between tangential walls of the neighbouring cambial cells. Changes in the location of the initials, as estimated from the arrangement of the confluent parenchyma cells forming interlocked grain in the wood of tropical rainforest trees, have shown that unidirectional intrusive growth of cambial cells between periclinal walls is responsible for changes in vessel orientation to the same extent. The aim of this study is a critical evaluation of assumptions being applied to cambial anatomy and a reinterpretation of the facts in the context of hypothesis that intrusive growth of the initials takes place between periclinal walls of the contiguous cells.

P0352. Phytoecological investigation of the sweet chestnut coenosis (*Castanea sativa* Mill.) in Belasitza Mountain - Bulgaria

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Progressively worsening of the health status of the chestnut phytocoenosis - drying - up, defoliation, development of semiparasites, parasites and diseases impose evolution of the degree of degradation processes and the factors responsible for this state.

In this investigation the plant species composition, structure of coenosis and intensity of cycling of some bioelements in chestnut coenosis in Belasitza mountain are present. They were used to evaluate the state of two types of model chestnut coenosis - century old seed chestnut stands and young sprout stands with different silvicultural management.

A total of 150 species of vascular plants were described. The structure of the studied phytocoenosis based on biological type, life form by Raunkier and floristic elements by Asov was determined. The assessments were made by the scales of Braun - Blanquet for abundance, coverage and sociability and by Ponjatovskaja for frequency.

The stores of mulch and litterfall are established and intensity of nutrient cycling of 8 elements were calculated.

P0353. The polymer network of wood cell walls - an examination of chemically degraded wood by X-ray scattering

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Wood cell walls are composed of complex interconnected polymer networks. The three structurally relevant groups of polymers are cellulose, hemicelluloses and lignin, of which cellulose is the one, whose function has been investigated most thoroughly. Our aim is to gain some more insight into the function of the matrix polymers, hemicellulose and lignin. For the present study, slices of Spruce wood (*Picea abies* [L.] Karst.) were degraded by three different chemical treatments: maceration by hydrogenperoxide, lignin removal by sodiumchlorite and swelling by sodiumhydroxide. Those treatments each produce a different effect on the condition of the individual cell wall polymers and on their composition. The corresponding structural changes of the cell wall were observed by small-angle scattering (SAXS) and wide-angle diffraction (WAXS), in the wet state and after drying.

P0354. The development of cell walls during wood formation in *Larix sibirica* Ldb. stems

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The changes in growth rates of primary and secondary walls and the accumulation of hemicelluloses in the course of wood formation in *Larix sibirica* Ldb. stems were studied. To study the growth rate the samples were isolated from tree stems in the course of season. The growth rates of primary and secondary walls in the zones has been shown to decrease during tracheid development. To investigate the accumulation of hemicelluloses in tracheid walls at different differentiation stages xylem cell layers from cambium zone, radial cell expansion zone, secondary wall development before lignification and two consecutive layers of lignifying cells of early-wood and three consecutive ones of lignifying latewood tracheids as well as mature xylem cells have been obtained from the stem cuttings of 20-year-old larch trees. The amounts of polymer carbohydrates, deposited at each of development stages were calculated per dry weight and per cell. Pectin, arabinogalactan, arabinogalactan-proteins, A and B hemicelluloses have been found to have own dynamics at the each of differentiation stages according to their functional role in the creation of wall structure and growth cell.

P0355. Comparative Wood Anatomy of Nepalese Ulmaceae

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Eleven wood samples of Nepalese *Ulmus*, *Celtis* and *Trema* belonging to the family Ulmaceae are collected from different localities and described their wood structure. Wood is ring porous, semi-ring porous or diffuse porous. Transition from early wood to late wood is abrupt. Early wood pores are one to three layered, tangentially arranged. Late wood pores are arranged in dendritic or ulmiform pattern. Perforation plate is simple. Inter-vessel pit is alternate. Fiber tracheids, tracheids and libriform fibers are the non perforated tracheal elements. Wood parenchymatous cell is apotracheal, marginal and paratracheal. Rays are homo or heterogeneous. *Ulmus* differs from *Celtis* in ray structure and crystal location. A tentative key is prepared to identify the species based on wood character.

P0356. Molecular changes during deformation of wood studied by Raman Microscopy

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To meet the natural demands of a tree, wood tissues are formed in various ways with different anatomical, chemical and physical characteristics. As a result the mechanical properties of wood differ widely. To gain insights into the stress-strain behaviour at a molecular level, Raman spectra were acquired during tensile testing. Molecular changes were monitored by following changes in Raman bands attributed to vibrational modes of characteristic functional groups of cellulose and lignin. In normal spruce wood the band at 1095 cm^{-1} , corresponding to the stretching of cellulose (C-O-C), was shifted towards shorter wavenumbers, demonstrating that the cellulose molecule was subjected to a uniform stress deformation. No shift occurred for the 1600 cm^{-1} band, assigned to the aromatic C=C stretching in lignin. Investigating wood tissues, differing in chemical composition as well as orientation of the microfibril angle (e.g. reaction wood), will help to understand their stress-strain behaviour and the micromechanics behind.

P0357. Deformation behavior of wood under stress relaxation as cellular solids

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When compressive load is applied to wood in a transverse direction, the wood has high deformation capacity (low stiffness) and a superior ability to absorb the elastic energy. These characteristics of the wood subjected to transverse compression are important when wood is used as a construction material. Those characteristics are mainly due to the elaborate geometrical arrangements of various macro-structural and micro-structural units that constitute wood. In this study, the stress relaxation properties of coniferous wood in a radial direction were investigated to clarify the relationships between the various structures and the deformation behavior of wood. In the research on the macro-structure, that is, the annual rings unit, we elucidated

that every annual ring is deformed individually and that when the strain of one annual ring becomes large, the strains of the others are recovered. In the research on the micro-structure, that is, the single cell unit, we elucidated that the shape of cells changes every moment at the early stage under stress relaxation. These behaviors are considered to be due to the viscoelasticity of wood as honeycomb cellular solids.

P0358. From cell wall architecture to wall modeling and back: a systems biology approach

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Plants produce 180 billion tons of cellulose per year, which has enormous commercial value in, for instance, wood. Cellulose microfibrils are crystalline aggregates of linear polymers of D-glucopyranosyl residues, linked in the $-(1\ 4)$ conformation. The architecture of microfibrils is cell type and developmental stage specific, and a determinant of mechanical wall properties. Using a systems biology approach, we have formulated a theory explaining how cells can determine microfibril angle. This fully mathematical developmental model quantitatively relates the deposition angle of CMFs to (1) the density of active synthases in the plasma membrane (N), (2) the distance between individual microfibrils within a wall lamella (d) and (3) the geometry of the cell (D). The model constructs all textures and predicts them, if [N], [d], and [D] are known. By predicting texture when a parameter is altered, the model is a design tool for production of plants with desirable traits.

P0359. Role of Cellulases in the Wood Formation

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Cellulases are the enzymes hydrolyzing internal 1, 4- β -glucosidic bonds such as found in cellulose and hemicelluloses. Plant cellulases form a multigene family composed of nine subfamilies that differ in enzyme structure and expression. Since no *in vivo* substrates of plant cellulases are known, the role of these enzymes in plant tissues is not understood.

In this work, we investigate the function of two cellulase genes, *PttCel9A1-1* and *PttCel9B*, that have been found in wood forming tissues in *Populus*.

Sequence analysis placed *PttCel9A1-1* in the subfamily of KORRIGAN, which has a membrane-anchored domain and is essential for cellulose biosynthesis. 36 cellulase genes have been so far identified in poplar genome, including a closely related gene *PttCel9A1-2*. *PttCel9B* was associated with subfamily V containing cell wall residing cellulases.

We have studied the expression of *PttCel9A1-1/2* and *PttCel9B* in 13 different tissues of hybrid aspen and analysed phenotype of *Arabidopsis* lines overexpressing *PttCel9A1-1* and *PttCel9B*. The data obtained indicate that the genes play distinct roles in the wood formation.

P0360. Enzymatic modification of primary and secondary cell walls - a tool for better insights into the mechanical functions of cell wall polymers

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The unique mechanical performance of (ligno)cellulosic materials are based on nature's strategy in designing plant cells with an assembly of stiff cellulose microfibrils and soft matrix polymers. From a materials science point of view it is mainly the unique combination of stiffness and toughness of the cell wall that made plants to widely used biomaterials and inspires new biomimetic fibre composites. Knowledge of the mechanical relevance of each of the cell wall polymers separately is fundamental and imperative for the understanding of plant tissue properties. However, to date this knowledge is only rudimentary without input about the interaction of the cell wall components.

On one hand, *Arabidopsis* hypocotyls were enzymatically modified *in vitro* to investigate the polymer interaction in primary walls. On

the other hand, mechanically isolated softwood tracheids were treated with enzymes to suppress the mechanical function of specific cell wall polymers in secondary walls. Micromechanical tests on hypocotyls and individual tracheids reveal the relevance of polymer interaction for the mechanical behavior of the entire primary and secondary cell wall.

P0361. Wood resin formation in slash pine trees of subtropical Australia: Genetic and environmental influences as revealed by tree ring ^{13}C and solid state ^{13}C NMR spectra

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There is little information about genetic and environmental determinants of wood resin formation for tropical tree species. The objective of this study was to quantify the genetic and environmental influences on wood resin formation in tree rings of a slash pine plantation in subtropical Australia, using both stable isotope and ^{13}C NMR techniques. This has highlighted the exciting potential and opportunities to use tree ring ^{13}C and solid state ^{13}C NMR spectra for examining the genetic and environmental influences on wood resin formation. Tree ring ^{13}C proved to be an effective, non-destructive tool to differentiate between good and healthy trees without wood resin defects, and those bad trees with a lot of wood resin defects. Both water and nutrient stresses and their interactions have been implicated in the wood resin formation. The wood resin formation appears to occur in the very dry growing season, where both water deficit and nutrient deficiency might be experienced.

P0362. Variation and relationship of tracheid length and S2 layer microfibril angle in *Cupressus arizonica* compression wood and opposite wood.

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In this study, variation of tracheid length and microfibril angle of S₂ layer (MFA) from pith to bark in compression and opposite wood of *Cupressus arizonica* was investigated and it was tried to determine the relationship between these two factors, quantitatively. Hence, four leaned stands were selected from the cypress plantation of Tehran University. After sampling and determining tracheid length, MFA was measured by two methods; namely "inducing cracks in the secondary wall" and "Orientation of pit aperture". There was a good agreement between these two methods which is described in the following equation:

$$Y = 7.3 + 0.87 X$$

X is the value of MFA obtained by the second method and Y is probable value which will be obtained by the first method.

Obtained results showed that tracheids are shorter and mean MFA is bigger in compression wood than opposite wood. Besides, in both tissues tracheid length has increased from pith to bark while MFA has decreased in the same direction. There was an intensive and negative correlation between these factors in both tissues. The best equation describing this correlation is:

$$L = 0.09 + 1.97 \text{ Cot}$$

L is the mean tracheid length and Cot is the mean MFA.

P0363. Contact and Non-contact Wood Fibers in Some Hardwoods

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Contact and non-contact wood fibers of *Pterocymbium beccarii*, *Paulownia tomentosa* and *Albizia julibrissin* were investigated. In the Mäule color reaction, wood fibers with small diameters (tips) appeared much darker in color than those with larger diameters (bodies), but there were no significant differences in the depth of

color between the contact and non-contact wood fibers with similar diameters. This observation indicates that the tips of the wood fibers tend to be richer in syringyl lignin than the bodies, but there is no positive correlation between the content of lignin and the distance of the wood fiber from the ray. In the immunogold labeling with anti-xylan mouse antiserum, no essential difference was found in labeling distribution and density between two types of the wood fibers. In *Albizia julibrissin*, moreover, almost all wood fibers regardless of contact or non-contact with the ray contained starch grains in the outer portion of the outermost annual ring. These findings suggest that even the non-contact wood fibers might be supplied with almost the same amount of photosynthetic products, which are a source of raw materials for cell wall biosynthesis.

P0364. Rope-controlled design

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Moving biological structures are highly optimized in terms of mechanical strength and minimum weight. Due to their materials strength properties, tension-loaded components are therefore often highly qualified for material-efficient and lightweight structures. Humans, for example, have about 650 muscles (ropes!) and only about 225 bones. We are a rope-controlled biological design. In the past, lightweight design was achieved in engineering by using the SKO method (Soft Kill Option), which simulates the merciless work of osteoclasts. Non-load-bearing members of a mechanical component are nibbled away in a computer simulation. However, leasing rates of FEM software (Finite Element Method) are expensive. For this reason, a computer-free method was developed which learns from the mammal skeleton and from tensile belts in plants. In the cases tested so far, this method yielded results similar to those of the SKO, but with much less effort. The poster shows "hidden tension ropes" in nature and how the "thinking in ropes" method can be used for designing lightweight technical components.

P0365. Plasticity of single wood fibres - Basic research on cold forming of wood

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At the molecular level of wood the cell wall polymers e.g. cellulose, lignin and hemicelluloses are the basic components influencing the mechanical behaviour of the tissue. However, only little is known about the mechanical interaction of the constituents. It was shown recently, that wood with a high cellulose microfibril angle in the S₂-layer shows permanent plastic deformation without significant mechanical damage of the matrix, such as usually only observed in metals. The gliding of dislocations is replaced by a molecular stick-slip mechanism operated by some sort of "velcro" connection. To better understand the mechanical interaction of the cell wall components, individual polymers were suppressed in their functioning. Single fibres were isolated mechanically and the cell wall assembly was modified using specific enzymes. Micromechanical tests were carried out to characterize the mechanical behavior of the modified material without the missing component and thus, to learn more about the mechanical relevance of the eliminated polymer. In the long-term our objective is to modify wood, making it suitable for cold forming processes.

P0366. Development of biomimetically optimised pultruded composite profiles with high levels of dynamic load capacity and high vibration damping

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The aim of the project is to transfer findings about fibre orientation of plants into technical composite profiles with optimised weight related bending stiffness and strength combined with high dynamic load capacity and damping.

Like in plant stems the fibres embedded into a foam-like matrix, will be thinned out in regions of the cross section with less stress. Furthermore, structural collapse caused by of high bending and

shear stresses between flexible matrix and stiff fibres will be avoided by gradual transitions of stiffness.

Additionally, a distinctive circular spacer structure will provide excellent bending and buckling stability. Furthermore T-shaped fibre bundles along the longitudinal axis contribute to high stability and vibration damping.

An optimal technical realisation of these principles could be achieved by means of the braid-pultrusion process. Specially designed braid-pultrusion can generate spacer fabrics, and produces „technical plant stems“ with the features described above, reproducing the principal optimisations of plant paradigms.

P0367. Microcapsules prepared from pollen grains are promising tools in biotechnology

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Several angiosperms and gymnosperms produce large amounts of pollen grains that are covered by a sporopollenin envelope with fascinating properties. Microcapsules representing isolated exines of different species are rigid and nearly monodisperse particles with excellent packing properties for high performance liquid chromatography. They have unique properties for their application as filtrable support particles in biocatalysis and bioanalytics. The extremely high resistance of sporopollenin to heat as well as to harsh acid and alkaline treatments allows simple sterilisation, purification and regeneration.

Sporopollenin microcapsules from pine, birch and walnut have been designed for a new type of size exclusion chromatography that enables rapid and efficient isolation of extremely large polymers from a polydisperse colloidal mixture. Based on this principle, there has been developed a system for DNA isolation. The miniaturised columns or column arrays enable rapid and complete separation of genomic DNA from the proteins of fresh crude cell-free extracts obtained from plant tissues, yeast or bacteria.

P0368. Biomimetic application of plant gaseous exchange

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Plant gaseous exchange by stomatal diffusion represents a tightly controlled process. The details of stomatal regulation are very complex and still not yet fully understood. Since the stomatal pore opens under high turgor pressure of the guard cells and closes if the turgor decreases, it is, however, possible to apply this basic mechanism to humidity-sensitive textiles. Biomimetic mimicking of stomatal regulation is therefore achievable by various structural concepts by combining different fibres and materials. Furthermore, there are many variations of the stomatal architecture which probably represent additional structural optimizations of stomatal diffusion. For example, it can be shown that a sunken stomatal position and cutinization of the stomatal channel can decrease stomatal conductance significantly. Detailed analyses of the biophysical effects of the various stomatal fine structures will increase 1) the biomimetic potential of this biological mechanism, and 2) our understanding of ecophysiological adaptations of stomatal architecture.

P0369. A microfluidic system based on Münchs theory

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The osmoregulatory principles of phloem transport first formulated by Ernst Münch are of high interest for microfluidics. A micropump has been constructed on this background. As in the plant, volume flow is driven by a stationary osmotic gradient created by continuous regeneration of the solute (upstream) and its dilution within a membrane-boarded micro-channel (downstream). The prototype uses dissolution of NaCl for solute regeneration and a vapour-permeable hydrophobic capillary membrane on the dilution path. It enables pulse-free flow in the range of 2 to 30 nl per second for several days. The volume delivered by the pump with an adjustable flow rate is about twenty times larger than that of the osmotic working cell. An increase of the back pressure by 1 bar reduces the flow rate by less than 2 %. The pump contains a mobile separation element between the salt solution leaving the working cell and the pumped liquid. Osmotic micropumps of the

described type and arrays of such pumps may be useful for labs on a chip, micro-electrophoresis, micro-injection and other applications, where small volume fluxes have to be manipulated.

P0370. Biomimetic Hydrophobic Surfaces generated by Self-Assembly of Plant Waxes

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On plant surfaces a great diversity of micro- and nanostructures exist. These structures are often built up by crystalline and self-assembled waxes, which form hydrophobic microstructured surfaces. A surface template effect of artificial surfaces (HOPG = Highly ordered pyrolytic graphite) was used to generate highly ordered biomimetic surfaces by re-crystallisation of wax tubules from Lotus (*Nelumbo nucifera*) and wax platelets, extracted from wheat (*Triticum aestivum*) leaves. These in-vitro systems of substrate and adsorbates give a well defined biomimetic interface for studying a wide range of surface interactions at the micro- and nanometre scale. The dynamic process of wax tubule crystallisation on HOPG was observed in real time by Atomic Force Microscopy (AFM), and the mechanism of tubule formation documented. The in vitro formation of wax platelets was analysed by scanning tunnelling microscopy (STM), AFM and scanning electron microscopy (SEM), and the organisation from the molecular level up to the three dimensional crystal is documented.

P0371. CYCLOIDEA and flower head development in Aster family

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Flowers of the aster family are arranged in a compressed inflorescence called a flower head (capitulum), which resembles a solitary flower. Most common flower head forms are radiate or discoid. Radiate heads contain disc florets in the centre, surrounded by marginal ray florets, whereas discoid heads have only disc florets. In *Senecio vulgaris*, the radiate and discoid head forms are controlled by a single locus, RAY. Linkage analysis reveals that two *Senecio* CYCLOIDEA genes (sCYCs) are linked to the RAY locus. These two genes are expressed only in ray florets, suggesting that sCYCs are involved in ray floret development. This suggests that sCYC is involved in generating dorso-ventral symmetry in a novel way that is different from *Antirrhinum* CYC. Generating transgenic *Senecio* plants with higher or lower levels of sCYC expression is underway to confirm the function of sCYCs in flower head development.

P0372. Study on floral biology and fruit setting in Pointed Gourd (*Trichosanthes dioica* Roxb.)

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The knowledge of floral biology helps a great deal in formulation of breeding strategy in any crop plant as it helps in crossing programme. With a view to studying the floral biology, Pointed gourd (*Trichosanthes dioica* Roxb.) of Cucurbitaceae, was taken as the experimental material. Characters like flower form, pollen shape and size, anthesis time, anther dehiscence, pollen viability, germinability, stigma receptivity and fruit setting under controlled pollination were taken into consideration. The opening of flowers and dehiscence of anthers tend to go together. Opening of female flowers almost synchronised with those of male flowers. Temperature had an effective role in development of buds, anthesis and dehiscence of anthers. Fruit setting percentage reduced with the aging of pollen. Staminate flowers developed earlier than the pistillate flowers. The average interval between the opening of the first and last flower within a same plant ranged from 2-6 hours.

P0373. Molecular evolution of floral dorsoventral asymmetry

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A key problem in evolutionary biology is the understanding of how genetic pathways diversify to give rise to a new morphology. Flower dorsoventral asymmetry has evolved multiple times

independently. In *Antirrhinum*, flower dorsoventral asymmetry requires the combined activity of four key transcription factors: *CYC*, *DICH*, *RAD* and *DIV*. *CYC* promotes dorsal petal and stamen identity through directly binding to *RAD* promoter. *RAD* antagonises the activity of *DIV* cell and non-cell autonomously restricting its activity to the most ventral part of the flower. In *Arabidopsis*, a species with radial symmetrical flowers, *TCP1* (*CYC* orthologue) does not interact with any of the six *RAD*-like genes. In contrast to *CYC*, *TCP1* expression is transient and cannot be detected in floral primordia older than stage 2. Persistent expression of *CYC* in 35S:*CYC*:GR *Arabidopsis* plants promoted petal growth. The network of genes regulated by *CYC* is being analysed. Our results suggest that the persistent asymmetric expression of *CYC* and the recruitment of *RAD* expression in the dorsal domain of *Antirrhinum* floral primordia were important for generating a flower with dorsoventral asymmetry.

P0374. Genes and functions activated by the homeotic gene *AGAMOUS* during floral organ initiation in *Arabidopsis*

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In *Arabidopsis*, the homeotic gene *AGAMOUS* (*AG*) terminates meristem activity and promotes development of stamens and carpels. To understand the program of gene expression activated by *AG*, we followed genome-wide expression during early stamen and carpel development. The *AG* target genes identified include most genes for which mutant screens revealed a function downstream of *AG*. Novel targets were identified and a core of genes that showed sustained activation is currently under study. The pattern of expression of these genes was analysed using *in situ* hybridisation confirming their expression in early reproductive organ primordia. The promoters of these genes are enriched for *AG* binding sequences and binding of *AG* was confirmed *in vitro*. We confirmed interaction with *AG in vivo* using chromatin immunoprecipitation for most of the other regulators of stamen and carpel identity (*AG*, *AP3*, *SEP3* and *CRC*) and for *GA4* (which suggested a role of *AG* in promoting gibberellin biosynthesis). These results suggest firstly a co-ordinated regulation of floral organ identity genes and secondly a role for gibberellins in floral organ initiation, which is currently under study.

P0375. Cellular and molecular mechanisms that lead to the unisexuality in flowers of *Opuntia stenopetala* (Cactaceae)

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The unisexuality is a mechanism that promotes the cross pollination. Some dioecious species never develop one of the sexual organs or its development is arrested. However, the cellular and molecular mechanisms that lead to the unisexuality are not well understood.

Our work is focused on the study of the dioecious cactus *Opuntia stenopetala*. We have found that male flowers show several defects in the ovary such as poor ovule development; lack of ovules and in some plants a total absence of the ovary cavity. Additionally, all the male flowers lost the stigmatic surface; instead, the style end is forming a sharp pointed structure. In order to understand the molecular defect associated to defective stigma phenotype, we have cloned from *O. stenopetala*, the partial cDNAs of *Tousled* and *Stylish*, two genes associated to this phenotype in *A. thaliana*. This analysis is in progress.

On the other hand, we have found male sterility in female flowers, since these plants do not produce pollen grains because during the anther development the microspore mother cells (MMC) do not go to or finish the meiosis, instead it we have evidence that MMC go to programmed cell death.

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P0376. Switching sex along the spike: synchronised monoecy in *Ecdeiocoleaceae*, sister of *Poaceae*.

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Ecdeiocoleaceae includes only two species, *Ecdeiocolea monostachya* and *Georgeantha hexandra*. Both are wind pollinated and monoecious; spikes produce zones of female and

male flowers, with several switches of sex in an acropetal succession. *Ecdeiocolea* commonly shows a sequence up the spike: female/male/female/male/female, with high fruit set in both of the lower female zones. *Georgeantha*, with fewer flowers per spike, shows fewer switches. Through most of the flowering season, all spikes on a plant flower synchronously, either all in a female or all in a male phase. Fruits are nuts in *Ecdeiocolea*; capsular in *Georgeantha*. The two species are found in semi-arid habitats in southwest Australia. Both are highly xeromorphic with leaves reduced to few sheaths and with stomates in deep grooves. *Ecdeiocoleaceae* floral morphology may assist in interpreting the morphology of grass flowers, but the monoecy and xeromorphy are presumably autapomorphic. There is robust support from DNA sequence data for the clade (*Poaceae*, *Ecdeiocoleaceae*, *Joinvilleaceae*) and some support for *Ecdeiocoleaceae* as the closest extant sister to the *Poaceae*.

P0377. Environmental control of sepalness and petalness in waterlilies

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The conventional concept of an "undifferentiated perianth" obscures the fact that individual perianth organs may be differentiated into sepaloid (sepal like) and petaloid (petal like) regions. We have observed such differentiation in genera from several families including the basal angiosperms *Nuphar* and *Nymphaea* (*Nymphaeaceae*). In *Nuphar* the perianth organs traditionally called sepals exhibit both yellow petaloid and green sepaloid patches that show anatomical distinctions in addition to color differences. In *Nymphaea* the perianth organs near the boundary between "sepals" and "petals" are often subdivided into sepaloid and petaloid regions, rather than being intermediate in morphology over their entire surfaces. Our data suggest that the environment of a perianth organ helps specify these regions. The traditional concept of "sepal" and "petal" includes a hidden assumption that we believe is false: that sepalness and petalness must refer to whole perianth organs. We suggest a novel theory of perianth evolution, in which differentiation of the perianth into sepals and petals was predated by the evolution of sepalness and petalness.

P0378. Assessing adaptive evolution in homeotic B MADS-Box genes. Implications in the evolutionary history of petal development.

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We investigate the evolution of petals at the molecular level by performing statistical tests to detect adaptation in the coding sequences of B MADS-Box genes, determinant in the molecular mechanisms of petal and stamen specification.

We use likelihood methods to evaluate the role of positive selection (PS) at specific moments during the evolution of B genes and at specific regions of the codified proteins, correlating our findings with crucial events in the evolutionary history of flowering plants. Our analyses focus on the AP3-PI duplication given its role in the evolution of heterodimerization and B function, and the eAP3-TM6 duplication and its possible role in the diversification of core eudicots.

We present phylogenetic hypotheses on which PS analyses were based, and report a complete description of substitution rates, divergence dates and sites under PS, particularly within the C-terminus of the amino acid sequences of core eudicots. Our results contribute to a better understanding of the evolutionary forces driving the functional diversification of B proteins, and provide a framework to explain petal evolution and core eudicots radiation.

P0379. Gene expression in homeotic flowers of carrot CMS plants

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Flower formation is predominantly controlled by the nuclear genome. As known from the maternally inherited trait of 'cytoplasmic male sterility' (CMS), extranuclear genetic information

also takes part in flower development. CMS flower defects are attributed to a perturbed interaction between specific nuclear (restorer/maintainer) genes and aberrant mitochondrial genes. The homeotic florets of the 'carpeloid' CMS-type of carrots (*Daucus carota*) resemble nuclear organ identity mutants impaired in function of certain MADS transcription factors. We observed a reduced expression of the B-class MADS genes during early organ differentiation of 'carpeloid' carrot CMS flowers. CMS flower alterations are presumed to be an effect of reduced respiratory efficiency or another mitochondrial dysfunction. To detect a stage-specific correlation to an impaired mitochondrial function, we have analysed the expression of mitochondrial genes including also putative small regulatory RNAs in normal fertile and homeotic CMS florets of carrot plants by RT-PCR and *in-situ* hybridisation.

P0380. Xylan deposition on secondary wall of fiber and tracheid

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Xylans, including glucuronoxylans and glucuronoarabinoxylans, are abundant hemicelluloses in the secondary cell walls of xylem tissue.

Immuno-EM study showed that xylan localizes exclusively in secondary wall layer of xylem cells. Labeling increased in deep cell wall layers when the layer close to plasmalemma was being formed, which was observed in both fiber of woody dicotyledonous species and tracheids of conifer. This result suggests that xylan continues to be deposited after cellulose microfibril deposition. FESEM observation coupled with chemical extraction also showed that xylan penetrated into cell wall and continuously accumulated on microfibrils.

Xylanase treatment after delignification caused extensive swelling of secondary wall. A transverse view of fiber cell wall showed rounded shape after xylanase while angular shape was observed in non-treated and delignified sections. Xylan might act as a cementing material for lamellated structure of cellulose microfibrils.

P0381. Structural analysis of linear hydroxyproline-bound glycosides in *Chlamydomonas reinhardtii*

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Extracellular hydroxyproline-rich glycoproteins (HRGPs) in *Chlamydomonas* and higher plants are posttranslationally modified by sugars O-glycosidically linked to hydroxyproline. While higher plant HRGPs of the extensin family are decorated by a simple mixture of tri- and tetraarabinosides, *Chlamydomonas* employs a broad variety of mixed, linear and branched sugar side-chains. As *Chlamydomonas* HRGPs are considered to be evolutionary ancestors of higher plant extensins, we were interested in the comparison of the respective glycosylation strategy based on anomeric conformation and linkage position of the sugars involved. We thus purified hydroxyproline-glycans isolated from *C. reinhardtii* HRGPs to homogeneity and analyzed the most abundant linear species by NMR and GC-MS after deuteromethylation. Our data proof that the *C. reinhardtii* linear hyp-glycans exclusively consist of arabinose and galactose, partly methylated in characteristic positions. Furthermore, we found that the first two arabinoses linked to hydroxyproline have the same anomeric conformation and linkage to each other as the arabinosides analyzed in higher plants.

P0382. Interaction analysis of outer wall HRGPs of *C. reinhardtii* and *C. incerta*

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The cell wall of the unicellular green alga *Chlamydomonas reinhardtii* exclusively consists of hydroxyproline-rich glycoproteins (HRGPs), some of which are crosslinked to form the inner wall onto which perchlorate-soluble outer wall HRGPs assemble, a process which is called nucleated assembly. A nucleated assembly was observed as well, when *C. reinhardtii* outer walls were assembled onto perchlorate extracted cells of *Volvox carteri*. Isolated perchlorate extracted outer wall HRGPs of *C. reinhardtii* self-assemble *in vitro*, a process that was shown to produce the same lattice organization as *in vivo*. In a recent study, we have used isolated outer wall GPs of *C. reinhardtii* and *C. incerta*, which

are capable to nucleated assembly and analyzed the interactions of their respective outer wall HRGPs under equilibrium and real time conditions. Interaction data of the respective species and chimera will be presented.

P0383. Nectar resorption in flowers of *Sinapis alba* L., Brassicaceae and *Platanthera chlorantha* Custer (Rchb.), Orchidaceae

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In the flowers of *Sinapis alba* nectar is secreted by two pairs of nectaries and accumulated as drops between filaments and in the cavity of sepals whereas in *Platanthera chlorantha* nectar is produced and accumulated within a spur. Previous studies of these species revealed that after a period of secretion and cessation, rapid nectar resorption occurs. The aim of this study was the observation of nectar resorption by the nectaries using radiolabelled sucrose. During the peak of secretion the nectar accumulated in unpollinated flowers was replaced with the same volume of labelled sucrose and after 12-48 hrs of incubation, at the resorption phase, parts of *S. alba* flowers with nectaries as well as fragments of *P. chlorantha* spur were sampled and fixed for microautoradiographic studies. In *S. alba* the presence of [¹⁴C(U)] sucrose was detected at the base of nectaries, in phloem elements of main vascular strands supplying glands, whereas both epidermis and nectary parenchyma showed no traces of radiolabelled sugars. In *P. chlorantha* the presence of labelled sucrose was stated mainly in the walls of nectary cells, which indicate an apoplastic route of reabsorbed nectar.

P0384. K⁺ channels regulate wood production

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Poplar cambial K⁺ content increases during growth period in a K⁺ supply-dependent manner. Upon K⁺ starvation or application of TEA⁺, a K⁺ channel blocker, the average vessel lumen and expansion zone area were reduced. Expression of three K⁺ channel types was analyzed. PTORK (release) and PTK2 (bidirectional) transcripts followed annual K⁺ variations in poplar branches. During dormancy both channels were low expressed but up-regulated during the wood producing season. KPT1 (uptake) was expressed in guard cells and buds, strongly correlated with bud break. PtKUP1 (a K⁺ transporter) was low-level expressed throughout the year, suggesting a housekeeping function. *Arabidopsis* petioles expressing PTORK- or the PTK2-promotor-GUS showed signals in vascular tissues only. In poplar, PTORK protein was detectable in young fibres, rays and phloem. Patch-clamp measurements identified PTORK like K⁺ release channels as the major K⁺ conductance of fibre cell protoplasts. A distinct K⁺ channel might be required for K⁺-dependent wood production and we now extend our analyses towards all poplar K⁺ channels. New findings regarding salt stress and ABA will also be presented.

P0385. Biometric study of charcoalfied softwood cross-field pitting

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Cross-field pitting is an important character for softwood identification. During charcoalfication, qualitative and quantitative modifications occur in cross-field pitting. These modifications may hamper the safe identification of some taxa. This work is a systematic biometric study of a wide range of gymnosperm cross-field pitting after experimental charcoalfication. We focused on the fenestrate, piceoid, taxodioid, cupressoid, araucarioid and podocaroid cross-field pitting types. Our main results are the following. (1) Cross-field pits of wood specimens dried out before charcoalfication are hidden by a thin closing wall; in this case, it is often impossible to discriminate the various types of cross-field pitting. (2) Piceoid cross-field pitting becomes taxodioid-like after charcoalfication. (3) Biometric study of charred softwood cross-field pitting dimensions shows that the ratios between height and width of pit aperture and border can be used to characterise four types of pitting (fenestrate, piceoid, taxodioid, cupressoid+araucarioid+podocaroid [= CAP]). The discrimination of the CAP type requires further investigation.

P0386. The Effect of Calcium on Wood Formation in Poplar

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Populus tremula L. x *populus tremuloides* Michx. clones were grown in hydroponics, supplied with Hoagland nutrition solution which was modified in its calcium contents. Energy dispersive X-ray analysis (EDXA) revealed an increase of calcium in the phloem, the cambium as well as in the xylem elongation zone with increasing Ca-supply in the nutrient solution. By using light microscopy a strongly reduced cambial zone of only a few cell layers in radial direction was shown under calcium starvation, accompanied with a limited elongation zone and early start of secondary cell wall formation. In addition, upon Ca²⁺ starvation transmission electron microscopy revealed significant changes in the cambial ultrastructure. Using FTIR technique on xylem cells formed under calcium starvation, we were able to detect a reduction of all absorptions, i.a. carbon-oxygen groups and of methoxy groups from S-lignin. On macerated fibres we found a significant reduction in the fibre length with decreasing calcium supply in the nutrient solution.

In conclusion, our results showed a significant influence of calcium on the structure and chemistry of young xylem cells.

P0387. The application of molecular markers for the identification of wood from different origins in the tropical tree family Dipterocarpaceae

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Dipterocarpaceae dominate low land rainforests and comprise the most important timber species in Southeast Asia. Illegal logging is a major cause of forest degradation in tropical rainforests. In order to develop tools for wood certification of dipterocarps, 200 wood probes will be collected from natural populations as well as from wood enterprises covering 20 geographic origins in 5 countries of Southeast Asia. DNA extraction methods will be adapted for marker analysis from wood. Different marker techniques - chloroplast and nuclear markers - that in former studies showed a strong differentiation between geographic origins will be applied. Specific PCR primers will be developed in order to amplify very short polymorphic regions also in degraded DNA probes from wood samples. These markers will be used for the certification (falsification) of the origin of wood from dipterocarps.

P0388. Aspects of the seasonal development of the secondary phloem in two Brazilian semi-ring porous trees

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The structure of the secondary phloem of *Cedrela fissilis* Vell. (Meliaceae) and *Citharexylum myrianthum* Camb. (Verbenaceae) was studied in specimens collected from a semi-deciduous seasonal forest in southeastern Brazil. Periodic collections were carried out from 1996 to 2000. Annual growth increments in the secondary phloem of these two species can be distinguished. In *Cedrela fissilis* early and late phloem are evident with one fiber band delimitating them. There is no distinct boundary between the early and the late phloem in *Citharexylum myrianthum*, but a gradual decrease in the diameter of sieve elements can be detected along each increment. The two species present very narrow sieve tube elements associated with their companion cells and phloem parenchyma cells, scattered along the outer surface of the cambial zone. The very narrow sieve elements mature just before the cambium dormancy that coincides with the dry season when the trees lose their leaves. These groups of very narrow sieve elements can be recognized as terminal phloem growth ring markers. The active cambium period begins with the wet season when trees leaf-out with the production of new phloem.

P0389. The International Association of Wood Anatomists

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Despite our modern, hi-tech world and a long history of the study of wood structure, there is a great demand for wood products and

research focusing on the anatomy of wood. The International Association of Wood Anatomists was founded in 1931 and has about 600 members in over 60 countries. The major aim of this association is to promote contacts between scientists from such diverse fields as wood development and ultrastructure, molecular developmental genetics of wood, archaeology, palaeobotany (including palaeoecology, palaeoclimatology), biomechanics, xylem physiology, systematic and ecological wood anatomy, dendrochronology, forestry, forest products, and wood technology. Other objectives are to exchange ideas, research material and information, to facilitate wood collecting, to promote the consistent use of terminology, and to stimulate research and publications in wood anatomy and related fields. The IAWA Journal is published quarterly and is the only international periodical entirely devoted to all aspects of fundamental and applied wood and bark structure. Further information is available from the IAWA website (<http://www.iawa-web.org>).

P0390. A novel biological rhythm of cell inclination change in cambium of *Cinnamomum camphora* T.Nees & Eberm.

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Figured wood in trees originates from cambium, in which the initial cells periodically change inclination. These oscillations represent the longest of known biological rhythms. Their period usually approximates 20 years. Here we introduce a short-term cycle discovered recently in a young cambium of camphor tree. We also give the first full description of its properties *in planta*. An analysis of young stems has showed that at the beginning of the ontogeny cambium produces remarkably straight-grained wood. However, in the 2-nd or 3-d year of cambial activity, in the middle of growing season, cambial cells start deviating to the right. Next spring they tilt to the left and, in a second half of the season, again to the right. No exception has been found from this sequence, although, theoretically, a mirror-like image of such annual cycle is possible. Two phases of the cycle are remarkably synchronised both circumferentially and longitudinally. A hypothesis has been put forward that the factor responsible for altering the direction of cell tilting in young stems is a peculiar, second flush of shoot apical meristem activity in the middle of growing season.

P0391. Climate sensitivity of tracheid wall thickness and ring width in intertropical trees and their relationship with cambial activity

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Two chronologies based on tracheid latewood wall thickness and ring width were obtained from *Abies religiosa* trees growing in the intertropical region of Mexico and their climatic sensitivity investigated by dendroclimatological techniques. In addition, samples of vascular cambium were removed over a two-years period to gain insight in cambial activity and seasonal wood development. Cambial activity is initiated at the end of January, reaching two peaks; one from March to April and the other during August; then activity slows down and ceases in mid-November. Two transition large periods, dormancy-activity and activity-dormancy, were observed within cambial activity cycle. Earlywood accumulation takes place from March to September and latewood from late September to mid-November. Tracheid wall thickness chronology shows low sensitivity whereas ring width chronology is positively correlated with precipitation in January and negatively with temperature in the May. The results suggest that rainfall in the transition period dormancy-activity and temperature after the first activity peak influence strongly wood accumulation.

P0392. The cambium activity in pernambuco-wood (*Caesalpinia echinata* Lam., Leguminosae)

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Caesalpinia echinata is a Brazilian endangered species. At first this species occurred throughout the Atlantic coast, but nowadays only a few populations remain left. We analyzed specimens planted in the state of São Paulo outside their natural environment as well as specimens planted in the state of Bahia in a natural area of occurrence. The periodic collections were carried out from 2000

to 2004. The cambium activity is different between the specimens of the two different areas. In the specimens of Bahia, the cambium activity is continuous along the year. The activity period is evidenced by the presence of fragmoplasts, recently formed tangential walls and xylem cells under differentiation adjacent to the cambium zone. On the other hand, the specimens collected in São Paulo present an active and an inactive period characterized by a sudden transition between the cambium zone cells and differentiated xylem cells. The cambium inactive period is related to a dry season which is, in general, five months long. In the specimens from Bahia, the cambium activity is continuous probably due to a shorter dry season.

P0393. Variability in cork oak wood anatomy in relation to water stress

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The variability in libriform fibers and rays (multi- and uniseriate) dimensions, and vessel size and distribution was studied in the wood of *Quercus suber* L. young trees, never submitted to cork harvesting, and growing in very extreme conditions.

Fiber and ray biometry was studied at three radial positions. Fiber dimensions presented an increasing radial trend, already reported for other *Quercus* species. Ray variables remained rather constant along the radius. Since ray dimensions in general decline with age, the observed stability was interpreted as an adaptation to improve water storage.

Vessels were measured continuously along the radial direction by image analysis, on transverse surfaces. Cork oak trees have smaller vessel sizes in comparison with ring-porous oak species. This feature was interpreted as an adaptation to the harsh edaphoclimatic conditions, resulting in lower rates of water conduction and lower probability of cavitation occurrence, therefore contributing to the species tolerance to water stress. The radial constancy of vessel density, not usual in other tree species, was also interpreted as a strategy to survive to water stress.

P0394. Wood anatomy of some Brazilian species, based on charcoal analysis. II. Compositae

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Application of wood anatomy is the foundation of palaeobotanical research, specially charcoal analysis (anthracology), which allows the reconstruction of past vegetation and climate, as well as palaeoethnological studies. Prior to the development of anthracological studies, it was imperative to improve our knowledge of wood anatomy, especially on charred samples. Wood structure is very well preserved after charring, even if some quantitative anatomical parameters may change. Charcoal identification is more effective, and methodologically easier, when ancient samples are compared to charred modern equivalents. Charcoal pieces were manually broken and the three fundamental wood sections examined under a reflected light microscope. Descriptions followed the IAWA list of microscopic features for hardwood identification. This paper presents descriptions of 17 species from 10 genera of Brazilian Compositae. Tribe Astereae: *Baccharis* (2 species); tribe Mutisieae: *Dasyphyllum* (1), *Gochnatia* (2), *Stiffitia* (1); tribe Vernoniaeae: *Eremanthus* (1), *Moquinia* (1), *Piptocarpha* (3), *Vanillosmopsis* (1), *Vernonia* (2); tribe Eupatorieae: *Eupatorium* (3).

P0395. Specialization of vessel elements in root, stem, and leaves of *Syagrus flexuosa* (Mart.) Becc. and of *Syagrus petraea* (Mart.) Becc. (Arecaceae)

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We examined root, stem and leaves of the suckering palm *Syagrus flexuosa* and of *Syagrus petraea*, a palm with subterranean trunk, which grow in the Brazilian cerrado. The cerrado (arboreal savanna) is characterized by a seasonal tropical climate, with poor and acidic soil. Cross-sections and macerations were used to analyze the material. Both species presented vessel elements whose width and type of perforation plate dimorphism corroborated what we know about the evolution of vessel elements in monocots. In relation to their length, we found that the vessel elements in the roots were much longer than those from the stem, differently from what the literature shows for monocots. Such differences bring to question the potential ecological and functional

roles of the specialization of vessel elements. It is possible that long and wide vessel elements at the roots would be related to the increase of water transport efficiency to the leaves. This would, in turn, replace the loss with transpiration and would avoid cavitation, common phenomenon in dry environments. Furthermore, we suggest that apart from avoiding embolism, these roots might also play a role in water storage.

P0396. The vertical migration of rays in cambium of *Aesculus turbinata*

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Storied pattern of ray distribution, called double storied pattern, in case of *Aesculus turbinata* cambium, develops very slowly and becomes visible as late as in 32-years old secondary xylem. In a young, nonstoried cambium, expanding rapidly, the total number of rays increases mainly in a result of splitting of already existing rays, which seems to act against the rising order. After such an event, however, the daughter rays vertically migrate on the cambial surface, with distances between their middle points increasing. This is facilitated by highly controlled, polar additions or (and) eliminations of ray initial cells on the opposite margins of each ray. The rays repetitively split and migrate until they reach a suitable position within the forming storeys of fusiform initials and this is how the double storied pattern is ultimately formed. Tracing the offspring of one ray often led to locations not in one or two but even three neighbouring storeys. The displacement of rays was also noted in older, 50-years old storied cambium indicating highly dynamic maintenance of the storied pattern.

P0397. Embolism potential of embryonic gas bubbles in conduits of vascular plants

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We analyze the final states (including embolism) of embryonic gas bubbles filled with water vapour, air, or both. They are created by air seeding or spontaneous vapourization. Their final states evolve from an interplay of surface tension, gas pressures in the bubble and xylem water pressure. The final states are calculated in terms of the initial values of bubble radius, xylem water pressure and number of air molecules.

The results are as follows:

(i) Pure water vapour bubble:

(a) If the initial radius exceeds a critical value the bubble bursts (causing embolism).

(b) If the initial radius falls below a critical value the bubble dissolves immediately after creation.

(ii) Air/water vapour bubble:

(a) If the number of air molecules exceeds a critical number (depending inversely on the xylem water pressure) the bubble bursts (causing embolism).

(b) If the number of air molecules falls below a critical value and the initial radius exceeds a critical value the bubble bursts (causing embolism).

(c) If both the number of air molecules and the initial radius fall below critical values the bubble attains a stable radius. In this case, the bubble can disappear by diffusion.

P0398. Fossil woods related with Malvales and Fagaceae from the Olmos Formation (Maastrichtian), Coahuila, México

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The Olmos Formation, in northern Mexico, bears a diverse biota with more than 80 plant taxa based on leaf and flower/fruit impression/compression fossils, and tons of permineralized wood waiting detailed study. Here, we introduce two wood types resembling the xylem of Malvales and Fagaceae. *Javelinoxylon*, previously described from the Cretaceous of Southern Texas, has storied axial and radial elements, septate fibers and scanty paratracheal parenchyma. The second wood type has heterocellular aggregated rays, diffuse in aggregates axial parenchyma (forming one cell wide bands) and vasicentric tracheids, suggesting resemblance with Fagaceae, specially with *Quercus* and *Lithocarpus*; however, the dominance of scalariform perforation plates, infrequent in Fagaceae, suggest the presence

of a new taxon. These woods document the earliest occurrence of storied structure, and what appears to be a member of Fagaceae with a major anatomical deviation when compared with its extant relatives.

P0399. Bark anatomy of *Meryta* (Araliaceae)

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Meryta comprises ca. 30 species distributed among the islands of Southwest Pacific, with highest diversity in New Caledonia. Bark structure was studied in 7 New Caledonian spp. (*M. coriacea*, *M. denhamii*, *M. lecardii*, *M. oxylaena*, *M. schizolaena* and an undescribed species), 2 from Samoa (*M. capitata* and *M. mauuluu*) and 1 each from New Zealand (*M. sinclairii*), Fiji (*M. tenuifolia*). The secondary phloem of all the species studied has axial secretory canals associated the aliform sheaths of the axial phloem parenchyma sometimes confluent into tangential bands. Two species, *M. sinclairii* and *M. tenuifolia*, differ distinctly by the total absence of sclerified cells in the collapsed secondary phloem (a condition never before noted in Araliaceae). This finding agrees with results from molecular phylogeny. Several features are of diagnostic value, incl. the presence and type of crystals in the phellogen and cortex, the type of secondary phloem rays, the occurrence of crystaliferous cells in collapsed secondary phloem, and the presence of radial secretory canals.

P0400. Wide-band tracheids in brazilian cacti

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Wide-band tracheids were present in species that had dimorphic and monomorphic wood. In all dimorphic woods in which WBTs were involved, the phase that contained WBTs was produced initially. In seedlings of *Cipocereus minensis* the first phase consisted of vessels and parenchyma in a matrix of wide-band tracheids and the second phase consisted of a fibrous matrix with vessels and parenchyma. In adults plants of *Opuntia leptocaulis*, *O. articulata* and *Nopolea cochenillifera* the first phase consisted of WBTs wood and the second phase of parenchyma matrix with solitary vessels. In the WBT wood of adults plants of *Discocactus placentiiformis* and *Melocactus ernestii* the axial cells differentiated into parenchyma that was distributed as small clusters between the vessels and wide-band tracheids (monophorphic WBT wood). In *Opuntia leptocaulis* and *Nopalea cochenillifera* the WBTs were sporadic, being detected in one or two vascular bundles of the same set of sections. In all species examined the bandlike secondary wall was present as annular rings or as a helix, but we observed "mixed" type or double-helix.

P0401. Presence of storeyed cambium in trees of the tropical rain forests: an adaptive strategy

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In a given environment, organisms capable of optimizing utilization of the resource available as a limiting factor, dominate. In tropical rainforests, light limits tree growth, during the first few years. The individuals that reach the canopy layer first, win the struggle for light. However, height of trees is limited by the mechanical properties of the trunk which depend mainly on its wood anatomy, which in turn depends on structure and activity of vascular cambium. Interlocked grain of wood provides high mechanical strength to the tree. Formation of interlocked grain is based on a fast cyclic change in orientation of the fusiform cambial initials, which is possible only in the storeyed cambium. It has been hypothesized that the presence of the storeyed cambium has an adaptive value for the fast-growing trees and forms an important evolutionary achievement which facilitates rapid cell rearrangement in the cambium without excessive expenditure of energy on events like elaborate apical intrusive cell growth, frequent oblique anticlinal cell divisions, and loss of the fusiform initials.

P0402. Floral development and presumed mycetomyiophily in *Tacca chantrieri*

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Flowers in *Tacca chantrieri* are bisexual, actinomorphic and self-fertile. Dull colours, filiform bracts, window-effects, a significantly higher temperature inside the flowers and a musty smell suggest the syndrome of mycetomyiophily. However, no pollinators were ever reported. Fungiform structures originate from both stamens and stigmatic lobes. Each of the six stamens has a hood-like shape resulting from connective and filament broadening and incurving. They form separate chambers with small lateral entrances hiding the pollen-sacs. The gynoeceum is epigynous. The free tips of the three carpels postgenitally fuse and completely fill the flower centre forming a large six-lobed structure. Diverse epidermal surfaces, furrows and projections are present in the flower, which are interpreted as guiding structures for pollinators. Contrary to previous interpretations we argue that the 5 mm large pollinators enter the flower by creeping below the stigmatic lobes. They first transfer pollen to the stigma and are then guided into the staminal hoods where they get dusted with pollen. Finally they leave the flowers through the lateral openings of the hoods.

P0403. Evaluating contrasting interpretations of reproductive axes in Pandanales using cladistic analyses of developmental gene sequences

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The newly-recircumscribed monocot order Pandanales now comprises five families: Cyperaceae, Pandanaceae, Stemonaceae, Triuridaceae and Velloziaceae; their precise relationships are uncertain because placement of the mycoheterotrophic Triuridaceae has hitherto proved problematic. As part of an ongoing developmental-genetic approach to resolving contrasting interpretations of the nature of reproductive axes in Triuridaceae (either as "true" flowers, or as highly condensed inflorescences, i.e., pseudanthia), we present a cladistic analysis of sequences homologous to selected transcription factor-encoding genes important in the mechanistic aspects of floral development (namely, LEAFY and APETALA3/PISTILLATA). The rationale for expecting incongruent cladograms from morphological, molecular non-developmental ('process-neutral') or molecular developmental ('process-laden') data sets, separately or in combination, will be discussed.

P0404. Embryology and seed development of *Lachnocaulon* Kunth (Eriocaulaceae)

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The embryology and seed structure of *Lachnocaulon* species were studied in order to describe the embryology of this north american genus and supply data for Eriocaulaceae's phylogeny. The anther in *Lachnocaulon* is bisporangiate and the 4-layered wall present a conspicuous and fibrous endothecium, and a secretory tapetum with uninucleate cells. Microsporogenesis is successive with isobilateral microspore tetrads; pollen is spiroaperturate and bicellular at release. The ovule is orthotropous, inverted, bitegmic and tenuinucellate, with a micropyle formed by the inner integument. The megagametophyte is of the *Polygonum* type, with a conspicuous antipodal cyst. The seed is endotestal, with a tanniferous endotegmen, and the seed coat is derived from the two ovule integuments. These embryological features agree closely with that known for Eriocaulaceae, and demonstrate the uniformity of the family. This investigation also indicates, as for several embryological aspects, the proximity of Eriocaulaceae to Xyridaceae, Mayacaceae, Rapateaceae, and Hydatellaceae (Poales), and they may be used to complement new databases in commelinids.

P0405. Sexual reproduction in a Mexican Caribbean seagrass: Description of fruits and seeds, and their potential distance of dispersión

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Seagrasses are angiosperms which complete life cycle in the sea. We studied *Thalassia testudinum* a dioic plant that occurs throughout the shallow coasts of the Western Atlantic. It mainly spreads through extension of horizontal rhizomes, but also high densities of flowers are reported. Not much is known concerning the role of sexual reproduction in population maintenance of this species. Development of the fruits and seeds was followed through histological techniques. In all cases the embryos developed from a fertilized zygote. The seed wall is thin and the endosperm is helobial. Early embryogenesis belongs to the Caryophyllad type, with a new variant *Thalassia* type. No radicle formation was detected instead a pair of adventitious roots were developed. The seeds presented cryptovivipary. During 2002, 92% of the fruits opened in situ, and the seeds dispersed on average 30 cm. The remainder of the fruits detached, and floated to the surface where they remained afloat for max. 10 d. and the distance of dispersal could be up to 100 km. In this population the sexual reproduction assure a mechanism for genetic variability and the connectivity between population.

P0406. Evolution of developmental genes and flower form in orchid diversification

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The objective of this project is to investigate how changes in the spatial and temporal expression of developmental control genes bring about new structures that are subject to natural selection and evolutionary fixation. In particular we are interested to find out how these morphological changes influence species diversification. Understanding species diversity is an issue of high priority, but not much is known about how it initially emerges. By investigating these problems in orchids, the largest family in the plant kingdom, we will provide novel insights into the relationship between the evolution of genes and morphological structures. MADS-box genes, encoding transcription factors, are the best understood plant developmental regulators. The results here presented are part of a experimental project to isolate and characterize the function and regulation of MADS-box genes in orchid flower development. We investigate the relationship between the phylogeny and functional divergence of these genes during evolution and the emergence of key floral features associated with reproduction and species diversification in the orchid subfamilies.

P0407. Floral structure in the basal grass genus *Pharus*

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Detailed knowledge of the reproductive structures of basal grasses is critical for understanding the homologies of grass reproductive structures, which are frequently anomalous with respect to other monocots. The tropical New World genus *Pharus* (5 species; subfamily Pharoideae) represents one of these basal grass lineages. Here we investigate floral structure in *Pharus* as part of ongoing comparative work on basal grasses. Most species have the classic monocot complement of six stamens (in male flowers) or staminodes (in female flowers), but in some representatives of two species (*P. latifolius* and *P. lappulaceus*) only four or five stamens or staminodes are present, reminiscent of another basal grass, *Anomochloa*. Examination of the vasculature associated with these reduced numbers reveals loss of the adaxial stamen of the inner whorl (5-staminate condition) and fusion of the two lateral adaxial bundles related to stamens of the outer whorl (4-staminate condition). Reduction in stamen number is correlated with adaxial compression of the flowers.

P0408. Diversity of climbers and lianas in North Andaman, India

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The climbers and lianas of North Andaman, India, are represented by 236 species under 127 genera and 53 families, including 3 species of ferns and 1 of *Gnetum* and monocotyledons with 30

species in 11 genera and 7 families. The most speciose families are Leguminosae (27), Convolvulaceae (21), Asclepiadaceae (16), Vitaceae (14), Menispermaceae (11), Dioscoreaceae (9), Arecaceae (8), and Smilacaceae (7). While 24 species are endemic, 89 are shared with mainland India and 123 have wider distribution in S.E. Asia. In the general pattern of species richness, 44 in the evergreen type, 38 in moist deciduous, 32 in mixed evergreen and 13 in littoral forests follow the semi-evergreen forest with 54 species. Bamboo thickets have 13 species and mangroves with only 7 species. Herbaceous climbers represent 71.61% of the total. Twiners and branch climbers are largest group (64.8%) followed by the tendril climbers (14.8%), sprawlers and scramblers (13.13%) and root climbers (7.2%). IVI ranged from 1.4 to 20.4 in the different forest types. Fruit is the most (60%) prevalent dispersal unit though anemochory (63.2%) supersedes zoochory (22.9%) and other dispersal means.

P0409. Fine-scale spatial variation in plant performance in a grassland: species-specific effects and neighbor root mass matter

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Many studies have shown that crowding intensity is an important predictor of plant performance in plant communities. Still in herbaceous communities aboveground plant biomass in the neighborhood is often a poor predictor of plant performance. We examine the hypothesis that belowground interactions and species composition of neighbors are more important for plant performance in such communities. We implanted phytometers of two species, *Anthoxanthum alpinum* and *Festuca rubra* into a mountain grassland, and examined their response to local variation in (i) aboveground biomass (ii) root mass in the soil, and (iii) abundance of individual species. The results show that neighbor root mass and by species composition were the the major determinants of phytometer performance. This suggests that interactions in the mountain grassland take place primarily belowground and that biomass-independent species-specific effects are important. This may be common in communities where maximum size of individuals is constrained.

P0410. Analysis of Aerenchyma Patterns in Aquatic Vascular Plants

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The existence of aerenchyma is considered as a important factor to distinguish aquatic vascular plants from other wetland species. We collected about 30 species of aquatic plants, *Potamogeton*, *Hydrilla*, *Vallisneria*, *Ottelia*, *Hydrocharis*, *Acorus*, *Sagittaria*, *Monochoria*, *Typha*, *Phragmites*, *Zizania*, *Scirpus*, *Eleocharis*, *Ceratophyllum*, *Myriophyllum*, *Oenanthe*, *Euryale*, *Nuphar*, *Nelumbo*, *Nymphioides*, *Polygonum*, *Persicaria*, *Trapa*, *Utricularia*, and *Limnophila*, from south Korea, and performed anatomical analysis to elucidate the characteristics of aerenchyma in aquatic vascular plants. As a result, aerenchyma patterns are divided into two groups. One is schizogenous aerenchyma that is a honeycomb-type or wide circular form, and the other is lysigenous aerenchyma that have cortical cells which are collapsed radially or tangentially. Especially in *Trapa*, aerenchyma is significantly enlarged in the petiole to give buoyancy to foliage, as well as to survive in flooding environments. Besides, we have cloned ADH gene of *Phragmites australis* and PRL gene of *Trapa japonica* to search genes associated with aerenchyma development in aquatic environments.

P0411. Determination mechanisms of matured plant construction in embryogenesis and germination in three species of *Araceae* Juss. family

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Development of the axial and lateral organs (phyllotaxis (P) formation, leaf (L) construction, branching (B)) during embryonic (EP), pregenerative (PP) and generative periods (GP) in species of genera *Anubias* (*An*), *Calla* (*C*) and *Amorphophallus* (*Am*) is studied. Starting from PP all the species has a regular B and either distichous (*C*, *Am*) or spiral (*An*) P. However the initiation of some first L after the cotyledon is not coordinated, resulted both in

irregular in divergence angle P (C, An) and B without any order, accompanied with death of buds (Am) and L tissue (An). By late EP or the seedling has rooted this processes put in order and L with blade similar to L in GP appeared. There is a partly repeating of this scenery as the adult plant B occurred. Analysis of the cell layers dynamic and structure using SAM and cytohistology methods has shown that determination of L and L part space position in EP lies in the establishment of species-specific space coordination between them without any respect to their rank. Such a coordination system is a topological invariant under following plant development. B and L complication conform to the increasing of this system fracture rank.

P0412. Ecomorphology and anatomy in *Sedum* species

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Growth-form and lateral growth was monitored in *Sedum album*, *S. rupestre*, *S. thartii*, *S. acre*, *S. sexangulare*, *S. maximum* (= *Hylotelephium maximum*) and *S. spurium* during one season. The 6 chamaephytic *Sedum* species show a mainly vegetative propagation. *S. maximum*, being a geophyte, is far less propagative. Within the first week of growing the primary root dies and is replaced by adventitious roots. Approximately after 12 weeks the stems of the young plants become prostrate and lateral shoots start growing. In *S. maximum* seedlings the older root-parts begin thickening after ca. 8 weeks. These root parts consist of a starch enriched storage parenchyma, which contains concentric fascicles with a central xylem. All species develop one or more libriform fibre rings during secondary stem thickening. Leaves are isobilateral. Mesophyll consists of peripheral rounded chlorophyll cells and inner isodiametric water-storing cells surrounding the fascicles. A distinct palisade parenchyma is not developed. All species increase their phytomass largely during spring.

P0413. Phenology and radial growth of trees in a subtropical rainforest of Mexico

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Association of *Aphananthe monoica*, *Pleuranthodendron lindenii* and *Psychotria costivenia* radial growth and phenology variables with environmental factors (local climate, vertical strata microclimate, and soil physical and chemical variables) was collectively evaluated. The generalized canonical correlation analysis (GCCA) revealed a strong influence of climate in phenology and radial growth. Leaf initiation and cambial activity periods were associated with maximum temperature and day length, and vascular tissue differentiation with soil moisture and rainfall. The first environmental canonical variable explained more than 84% of variance in the phenology and radial growth data in the species. The GCCA also allowed stating a general model of relationships among tree phenology and radial growth with climate, microclimate, and soil factors. However, after the individual species analysis, it was detected different relationships magnitude for each species, suggesting that each one take advantage in a different way of the environment in which they are growing, distributed throughout the different vertical stratum, and allowing them to coexist.

P0414. Leaf structure in *Chlorogalum* and its allies - Hyacinthaceae or Agavaceae?

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The North American genera *Chlorogalum*, *Camassia*, *Hastingsia* and *Schoenolirion* have traditionally been placed in Hyacinthaceae-Chlorogaloideae, but recent analyses of molecular sequence data have indicated closer affinity with Agavaceae. Here we investigate comparative leaf anatomy of Hyacinthaceae and Agavaceae as part of the *Anatomy of the Monocotyledons* series. Leaf morphology is frequently xeromorphic in these families, with some exceptions. Many Agavaceae have thick fleshy leaves, a character that was formerly used to group them with other xeromorphic Asparagales. Raised 'pustules' or dark-coloured spots are a feature of leaves of some Hyacinthaceae, which typically possess relatively little sclerenchyma and copious mucilage derived from large, 'hexigenetic' lacunae that are formed by the breakdown of large central mesophyll cells between the leaf

vascular bundles. Such lacunae are an unusual feature that is also present in Chlorogaloideae; they may allow rapid leaf expansion in deciduous-leaved species from xeric habitats. On the other hand, Chlorogaloideae share some features with Agavaceae, including the presence of styloid crystals.

P0415. Population Biology of an annual psammophytes of the South Russia

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Different species of plant, which followed to the determined ecological group and grew into similar ecological-coenotic conditions, are distinguished current models of shoot-forming. Some aspects of plant population biology are considered: 1) analysis of features of the morphological constructions of shoot (architectural models) and 2) correlation between morphological construction, ecology and phytocoenology of certain plant species. Some species of annual psammophytes of the Rostov region were investigated by methods of population biology. Researched populations of annual psammophytes had different architectural models of shoot-forming and grew on diverse sand areas. Classification of different species, comparison populations of one species growing in different sand areas were implemented, and diversity of architectural models within current taxon were investigated.

P0416. Floral correlations in two distylous *Primula* species

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An important question in pollination biology is that the sizes of flower parts are developmentally or functionally related to each other. Five floral traits (corolla tube length, anther and stigma height, stigma-anther separation, and anther length) were measured in long-styled and short-styled flowers of *Primula vulgaris* and *P. veris* in order to test these two hypotheses. The strong positive correlation between anther height and corolla tube length can partly be explained by developmental constraints, since anthers are fixed directly onto the corolla tube. According to the selective correlation hypothesis, stigma height is expected to be related to corolla tube length strongly in long-styled and weakly in short-styled flowers, but our results demonstrated weak correlation in both morphs. The two mating types varied considerably in the correlation between stigma-anther separation and corolla tube length: strong positive correlation was detected in short-styled, and weak negative correlation was detected in long-styled flowers. This phenomenon could be a possible explanation for the longer corolla tube in short-styled than in long-styled flowers.

P0417. Anatomical Studies in tribe Cereeae (Cactoideae, Cactaceae)

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The anatomy of species of *Arrojadoa*, *Brasilicereus*, *Melocactus* and *Stephanocereus* is here presented. These genera belong to tribe Cereeae and occur in the "campos rupestres", "caatingas" and cerrados (Brazilian savannah) of the Espinhaço range in the Northeastern region of Brazil, which represents the tribe centre of diversity. The anatomy of the dermal, fundamental and vascular tissue systems were studied in the genera, aiming to discover potential phylogenetic characters in an anatomically poorly studied group. The stems were transversal and longitudinally sectioned. Potential phylogenetic characters regard presence of crystals in the epidermis and hypodermis; shape of the lignified cork cells; type of hypodermis; occurrence of mucilage cells and sclereids in the cortex; lignification of the vascular cylinder; fibrous and non fibrous wood; presence of non lignified parenchyma; type of axial parenchyma; and presence of vascular tracheids in the secondary xylem.

P0418. A Survey of Root and Shoot Adaptations of Aquatic and Amphibious Angiosperms

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Roots and shoots of a wide spectrum of species of aquatic and amphibious wetland flowering plants were surveyed to determine the range of structural adaptations to aquatic and wetland

conditions. The majority of the wetland adaptations usually included the presence of aerenchymatous tissues, an endodermis and a secondarily lignified hypodermis, which also included an exodermis. These modifications are possible factors in internal ventilation, the exclusion of soil toxins from the roots and rhizomes, and structural support for either emergent or floating plants. We include representative members from orders such as the Nymphaeales, Acorales, Alismatales, Poales, Asparagales, Commelinales, Zingiberales, Caryophyllales, Lamiales, Ranunculales, Myrtales, Proteales, and Asterales to illustrate these adaptations, which included cell types and arrangements in the cortex, methods of producing aerenchyma, and the barrier layers.

P0419. Stomatal Architecture And Evolution in Basal Angiosperms

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Stomatal architecture—the number, form, and arrangement of specialized epidermal cells associated with stomatal guard cells—of 46 species of basal angiosperms representing all ANITA grade families and Chloranthaceae was investigated. A sample of 100 stomata from each specimen was coded for stomatal type and five other characters contributing to stomatal architecture. New stomatal types are defined, and many species are examined and illustrated for the first time. Character evolution examined in light of the ANITA hypothesis and evidence from the literature on fossil angiosperms and other seed plant lineages suggest that the ancestral condition of angiosperms can be described as anomostephanocytic, a system in which complexes lacking subsidiaries intergrade with those having weakly differentiated subsidiaries forming a rosette. Tangential divisions of these cells led to the profusion of different types in early fossil angiosperms and Amborellaceae, Austrobaileyales, and Chloranthaceae, while the state in Nymphaeales is little modified. Formation of new, derived types by tangential division appears to be a recurrent theme in seed plant evolution.

P0420. Rhizophore in Angiosperms

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Roots, stem and leaves are present in all vascular plants: pteridophytes, gymnosperms and angiosperms. Rhizophore are shoots that grow downwards with roots in the apex and that occur in living *Selaginella* and Carboniferous *Lepidodendrales*. More recently, rhizophores were also observed in angiosperms. This organ is a stem with positive geotropism and is the sole organ that gives rise to roots in the plant. It can be composed of nodes, internodes, and cataphylls protecting lateral buds (*Vernonia* L., *Cordyline* Comm., *Smilax* L. and *Curcuma* L.) or it can lack nodes and only present adventitious buds (*Rhizophora mangle* L. and *Dioscorea* L.). The rhizophores grow by apex expansion, without terminal buds. In *R. mangle*, there is a periderm similar to a root cap in the apex. In all species mentioned above, the rhizophore has exogenous origin, wide pith, polyarch stele, vascular tissues making up collateral bundles, endarch protoxylem and branching sympodially.

P0421. Architecture of blackberry (*Rubus hirtus* Waldst. & Kit. agg.) and the structure of chosen populations of this species.

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In forest there are periodic changes in resource availability. The blackberry, a common forest understory species, proved to be very sensitive to changes in environmental conditions. It can occur as a very common species forming a dense shrub layer or can survive for several years under deep shade, though often as stunted individuals. The expansion of blackberry in the first year after a gap creation is mainly by increase in cane length, and in the next years by the increase in density of population. Changes in cane length and changes in population density are very tightly interrelated. The most important factor of blackberry population dynamic is the probability of creating a long primocane (longer than 50 cm) by an individual. Most (usually more than 70%) of long primocanes are able to multiply by tip-rooting. This probability is modified by environmental conditions. This process seems to be a reaction to the regeneration cycle of natural forest.

P0422. Effects of the structural importance of roots of tropical trees on their conductive physiology.

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Previous studies have shown that roots have a high area specific conductivity compared to e.g. the stem. Tropical trees can develop an efficient conductive system with fewer large vessels and thus thin roots. Since these will have little mechanical strength, however, one might expect mechanically important areas of the root system to produce relatively more fibres or more and smaller vessels, and so have a lower area specific conductivity.

We measured the conductivity and studied the anatomy of different types of roots from four species of tropical trees, two with and two without buttresses. In buttressed trees the distal, superficial roots had a significantly higher area specific conductivity than the structurally important sinker and buttress roots. This was reflected in differences in vessel frequency as well as size. The superficial roots of non-buttressed trees, which also have a mechanical role, had a low conductivity. These and structural roots had a lower area specific conductivity than the stem. Hence the relative conductive physiology of different sections of the tree is more complex than previously thought and may depend on their mechanical functions.

P0423. Patchy nitrate availability alone contributes to heterogeneity in structure and functions in plants with contrasting vascular architecture

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Previous research has shown that patchy nutrient availability will generate aboveground heterogeneity in plant growth and chemistry, especially in plants where the movement of solutes from root to shoot is restricted by their vasculature. However, it is unclear whether nitrate itself generates this response when interactions with other nutrients are minimized. Using two broadleaf tree species, *Acer rubrum* and *Betula papyrifera*, grown in split-root hydroponics chambers, we examined the effects of patchy nitrate availability on morphological and functional traits of the plants. A comparison of plant parts with and without direct access to nitrate reveals that a higher nitrate supply can lead to larger leaf area, greater root proliferation, longer leaf lifespan and higher chlorophyll content. Data suggest that patchy nitrate availability can be responsible for heterogeneity in plant development and chemical composition, and is especially pronounced in plants with strong vascular restrictions in the movement of xylem sap.

P0424. Variation in the stomatal limitation of photosynthesis during vegetative phase change in *Metrosideros excelsa*

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Ontogenetic changes in leaf anatomy are a common feature of many higher plants, but the functional significance of this vegetative phase change is unclear. In *Metrosideros excelsa* (Myrtaceae) phase change is characterised by a progressive increase in leaf pubescence from juvenile to adult leaf forms. We measured stem hydraulic conductance and leaf gas-exchange in juvenile and adult leaves of *M. excelsa*. The stomatal limitation of photosynthesis (I) is $1-A/A'$, where A is the rate of net photosynthesis at an ambient CO_2 of 38 Pa, and A' is the rate that would occur if the concentration of CO_2 in the intercellular airspace (C_i) was 38 Pa (i.e. if stomatal conductance was infinite). In juvenile (glabrous) leaves of *M. excelsa* the stomatal limitation of photosynthesis is approximately 0.5, while in the pubescent adult leaf I is 0.27. This change in I is accompanied by a decline in stomatal density and an increase in leaf specific hydraulic conductivity. We suggest that in *M. excelsa*, leaf vegetative phase change reflects an increase in the capacity of the stem and roots to supply water to the aerial parts of the plant.

P0425. Rhizophore in Angiosperms**N. Menezes;**

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Roots, stem and leaves are present, with some exception, in all vascular plants: pteridophytes, gymnosperms and angiosperms. Rhizophore are vegetative organs which grows downwards putting out roots, occurring in the genus *Selaginella* and *Carboniferous Lepidodendrales*. All the characteristics considered exceptions in roots define rhizophore as a stem system: sympodial branching of exogenous origin, wide pith, polyarch stele, endarch protoxylem and vascular tissues making up collateral bundles. In Angiosperms was observed shoots growing with positive geotropism, which can store nutritive material. These organs are formed by nodes, internodes and cataphylls protecting buds and can originate adventitious roots. Comparative analyses were made in species of *Vernonia* L., *Dioscorea* L., *Smilax* L., *Cordyline* Comm., *Curcuma* L. and *Rhizophora mangle* L. In *R. mangle* the rhizophores grow downwards and it's apex is protected by a periderm similar to the root cap.

P0426. Procambium distribution and anisotomous branching in *Selaginella***E. M. Gola¹, J. Jernstedt²;**¹Institute of Plant Biology, Wrocław University, Wrocław, Poland,²Department of Plant Sciences, University of California, Davis, CA, United States.

Factors controlling the interrelationships of phyllotaxis, stelar type, and shoot branching are especially poorly known in the lycophyte lineage. The vascular systems of *Selaginella kraussiana* and *S. martensii* consist of two parallel steles (meristemes), which are supported in an air chamber by trabeculae with Casparian strips on radial and transverse walls. This results in potential physiological isolation of each meristeme. Experiments with dye microinjection into and dye diffusion throughout the vascular system show differences in supply to microphylls lying in lateral parts of the shoot, and to alternating individual anisotomous branches. The regular pattern of procambium distribution during dichotomous branching and the discrete nature of the two meristemes may be associated with sectorial transmission of morphogenetic signals. Anisotropy of morphogen distribution is a hypothetical mechanism to control anisotomous branching in *Selaginella*.

P0427. Dependence between the degree of changes in normal development of stem conducting tissues and assimilative leaf area of silver birch (*Betula pendula*)**N. N. Nikolaeva, L. L. Novitskaya;**

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Investigations into silver birch forms with normal wood (common birch) and abnormal wood (patterned and wavy birches) have lead us to a conclusion that structural abnormalities of conducting tissues in woody plants can be induced by high sucrose concentrations.

Sucrose is supplied to the trunk from green leaves. We investigated the foliage structural and functional characteristics in common, patterned and wavy birch trees.

The dynamics of shoot and leaf surface area growth, total leaf area of different shoot types, leaf dry mass per unit leaf area, foliage weight and leaf surface area per unit trunk weight have been studied. The common birch and patterned birch differed notably in most of the parameters considered, and wavy birch occupied an intermediate position. The data suggest that the foliage of patterned birch obviously produces more sucrose, which influx to trunk is the cause of abnormal development of conducting tissues. Experimental stimulation of the leaf surface area development in young common birch plants caused tissue abnormalities typical of patterned plants.

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P0428. The plant cuticle - a role for mechanical stability?**H. Bargel, C. Neinhuis;**

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The cuticle is a natural composite built of the biopolyester cutin, lipids, and variable amounts of cell wall fibres. Phenolics and a second polymer named cutan are frequently abundant. The main

function is limitation of water loss, while it acts as a multifunctional protective interface between plants and the environment. Evidence is high that it also adds mechanical support for tissue integrity and organ stability. In tomato fruit, the cuticle becomes increasingly important as a structural component during fruit maturation in addition to the epidermis. In an analogous way, the cuticle of leaves and primary stems of several species of different life forms and from varying habitats were mechanically characterized. Depending on the species, the results indicate that the cuticle seems to inherit a considerable role as a mechanically stabilising component in the plant's 'blueprint' in addition to the epidermal cell wall and sclerenchymatic fibres that has been largely neglected to date.

P0429. Biomorphological adaptations of the amphibious plants in Apiaceae family**S. E. Petrova, R. P. Barykina;**

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Life forms and structural adaptations of 5 amphibious plants from Apiaceae have been studied. 5 life forms have been identified: perennial polycarpics with adventive roots only, same with rhizomes, stolons, pseudoannual and winter annual plants. Various life forms are typical of *Oenanthe aquatica*, which could be winter annual, pseudoannual or perennial plant. The ability to produce water and terrestrial ecoforms is a distinctive feature of *O. aquatica*, *Sium latifolium*, *Cicuta virosa*. Plasticity of plant's structure, depending on the extent of submergence in the water, is typical of *O. aquatica*, *S. latifolium* and is manifested in heterophylly, forming short or long internodes, orthotropic or plagiotropic axes, in morpho-functional differentiation of adventive roots. Numerous stolons of *Berula erecta* serve for fast vegetative reproduction. Anatomical specialization, such as forming aerenchyma and reduction of mechanical tissues in the submerged organs of plants, emergence of the storage organs, such as rhizomes of *C. virosa* and adventive roots of *S. sisaroidium*, high content of toxins are also the distinctive features of studied amphibious Apiaceae.

P0430. Applying mutant analysis to the interpretation of the fossil record: A new perspective on leaf evolution**H. L. Sanders, G. W. Rothwell, S. Wyatt;**

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From the fossil record we can see a transformational series in leaf form which illuminates critical steps that may have evolved in parallel in leptosporangiate ferns and seed plants. Both clades appear to have leaves that are derived from axes of the most ancient vascular plants. Studies of leaf development in model species, such as *Arabidopsis*, have identified a network of regulatory pathways. Large scale changes in leaf morphology can be effected by modifications in a relatively small number of developmental genes. Therefore, these same genes could provide clues to the evolution of leaves from axes. Alterations in the development of mutants with abnormal leaves can be correlated with steps in the transformational series of leaf evolution revealed by the fossil record. In this study, building on previous work, the hypothesis tested is that the same developmental mechanisms were recruited and modified independently in each clade. Taxa with characters that reflect steps in leaf evolution are being studied to identify the pattern of evolution for leaves of each clade.

P0431. Using L-systems and Markovian models to simulate multi-scale phenotypic differences between apple tree cultivars**M. Renton¹, C. Godin², Y. Guédon², E. Costes¹;**¹UMR BEPC, Montpellier, France, ²UMR AMAP, Montpellier, France.

An approach for modelling and simulating the architectural development of apple trees is presented. The approach is based on using an L-systems framework to integrate local-scale Markovian models of terminal and axial bud fate at tree level. These models represent either branching patterns observed at node level along annual growth units or the succession of growth units along axes. Model parameters were estimated from architectural data collected on two different cultivars, Fuji and Braeburn. The integration of these models enabled us to simulate the development of the whole tree representing its topology at different scales. Tree simulations were used to evaluate what degree of complexity is needed in the local-scale models to capture emerging properties at the tree level, such as the number and distribution of fruits in the tree. We then investigate how this

approach can be used to give insight into the way that phenotype varies between different genotypes.

P0432. A biophysical growth model for root tips. Concentration gradients of auxin and cytokinin to focus zones of expansion and gravitropism response.

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Root growth and its response to external stimuli are mediated by hormones, auxin and cytokinin. We present a biophysical model for root growth, which relates the distribution of the two hormones to that of turgor, osmotic potential and wall extensibility, assuming a one dimensional string of cells. The hormone equations were given by a combination of diffusion, production, degradation, dilution and polar transport while the growth equations were derived by combining the Lockhart- Equation and a water influx equation. The model was successfully applied to characterize the distribution of relative elemental growth rate (REGR) under changing auxin concentrations. The implications of the new model are discussed in light of the distribution of gravitropic response that was recently revealed with high spatio-temporal resolution. We observed that curvature production occurs in three zones, i.e. the meristem and the zones apical and basal to the elongation maximum. Downward curvature was mostly found basal, whereas over one third was in the meristem. Interestingly, the presence of upward curvature was detected in the apical zone.

P0433. Real mutations in simulated architecture: implications for architectural evolution

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Genetic mutations such as lateral suppressor and self-pruning generally have obvious effects, either on inflorescence architecture on a whole-plant architecture. I examine the architectural variation that can be generated by known architectural mutations when incorporated into a rule-based, meristem-centered architectural simulation which is similar to a biologically elaborated L-system. I compare this variation with observed architectural variation within and among tomato species. Comparisons include visual similarity as well as standard quantitative architectural descriptors: height, order profile, path-length statistics, and metamer-demographic statistics. I suggest that the large-effect genes identified in mutant screens cannot account for subtler changes during architectural evolution, and I use this project to motivate a larger project on the computational inference of branching rules.

P0434. What makes floral phyllotaxis in *Magnolia* diverse - a lesson from virtual garden.

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Exceptional diversity of floral phyllotaxis in ancient *Magnolia* plant has remained a mystery for a long time. Among many patterns, spiral and whorled, some are very unique, hard to encounter elsewhere. Also they transform frequently, one type into another, along the generative axis. This indicates much greater intricacy of phyllotaxis in *Magnolia* than in any other plant, with no known explanation of this fact. Simulating process of floral parts initiation by floral apex with a computer program, being improved version of Battjes' program Phyllotaxis, we were able to produce very different phyllotactic patterns by changing primordia size at different rates. It is known that in real apices the size of primordia is changing with their identity twice: first between large tepal and small stamen primordia and then between small stamen and slightly bigger carpel primordia. Accumulation of geometric effects of these fluctuations leads to unpredictable final effect and results in diverse floral phyllotaxis. However, it is still not known why, with such seemingly random process, individual magnolia trees give a priority to some phyllotactic patterns over the others.

P0435. Roles of Determinancy and Specification of Meristem Identity in *Pisum sativum* Inflorescence Architecture

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We are modeling genetic regulation of *Pisum sativum* architecture, focusing on meristem determinancy and meristem identity. *UNI* (*PsLFY*) and *COCH* have quite global roles in regulating determinancy, while *DET* (*PsCEN*) appears to be limited to inflorescence meristems. Axillary bud and bract suppression in inflorescences depend on *PIM* (*PsAP1*), *BRAC* and *COCH*. Floral meristem identity is regulated by *PIM*, *BROC*, and *VEG1*. Gene interactions provide insight into pathways regulating inflorescence architecture. Double mutants of *pim* and the most severe allele of *coch* fail to flower. Double mutants of *pim* and other *coch* alleles flower in some early flowering time backgrounds. True terminal flowers form in *det veg-1* unlike WT. Plants homozygous for *uni* have a *det* phenotype, indicating that *UNI* is needed for *DET* expression. We are using a candidate gene approach to map and clone inflorescence architecture genes with phenotypes. Relative quantification of *PsAP1*, *PsCEN*, and *PsLFY* transcript levels in isolated WT, *uni*, *det*, *veg-1*, and *coch* meristems is being used to test and modify our model for the regulation of pattern in pea inflorescences. (Supported by NSF 0422840)

P0436. Visualization of experimental data using 3-dimensional computer models of developing barley seeds

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Three-dimensional computer models of developing barley caryopses at anthesis (1), at a stage showing syncytial (2) and cellularized endosperm (3) were constructed on the basis of series of thin cross sections. Caryopses were embedded in plastic resin and sectioned completely. High resolution digital images of all sections were taken. 3-D model building was carried out with commercially available software on a Windows® system. At first images were aligned according to their original position in the seed. In the following procedure all pixels in each of the images were assigned to the tissues they represent, e.g. the areas showing endosperm, embryo, integument. After all discernible morphological structures had been labeled in the images, the 3-D models were generated.

Sections from independent caryopses of comparable developmental stages were used for *in-situ*-hybridizations and immunohistological experiments. Experimental data were integrated into the models and spatial visualization of the expression patterns achieved.

P0437. Equivalent genetic regulatory networks in different contexts recover the spatial cellular patterns in the Arabidopsis root and leaf epidermis: a dynamical formal model

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A qualitative model of the gene networks that regulate cell spatial patterning in *Arabidopsis thaliana* root (trichoblast vs atrichoblast) and leaf (trichome vs non-trichome) epidermis is used to explicitly test if equivalent dynamic genetic systems may yield contrasting cellular spatial patterns when subject to contrasting signalling contexts. We reduced networks derived from experimental data into Turing-like reaction-diffusion systems for leaf and root networks and modeled them under different cellular contexts assuming both fixed and growing spatial domains. In all cases tested we obtained patterns that closely resemble observed *Arabidopsis* root hair and leaf trichomes spatial patterns. Our results suggest that equivalent gene networks may create different spatial cellular patterns under the influence of contrasting cellular contexts in *Arabidopsis* epidermis, and that this may be the case in other biological systems. This study contributes to a general understanding of network structure, function and evolution interplay and pattern formation in living organisms.

P0438. Phyllotaxis - an e-learning unit for beginners and lovers

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What does the shoot apical meristem (SAM) do and how does it do it? Phyllotaxis is the mode of arrangement of leaves, scales, or bracts with flowers along the plant stem. An e-learning unit is now available on CD in English and German (ISBN 3-905677-06-7).

Beginners will understand some basics of regular pattern formation in vascular plants. Computer animations allow the user to admire virtual plants with most phyllotactic patterns observable in nature, including transformations between frequent patterns (e.g. Fibonacci systems) and rare ones (e.g. Lucas). These animations are based on graphic tools by O. Deussen et al. (see www.computerpflanzen.de). The presentation of unique and rare phyllotactic patterns (including exceptions to Hofmeister's rule) allow improved estimation of the developmental control of phyllotactic pattern formation. Students in plant systematics, biomathematics and developmental biology should not miss this elegant and simple introduction into a fascinating and puzzling topic of botany.

P0439. The photosynthetic response of heat-induced electrical signaling in poplar

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Plants respond to various abiotic stimuli by generation and propagation of electrical signals. To get more information on the response of photosystem II (PSII) in higher plants, we investigated heat-induced long and short-distance signalling in poplar trees (*Populus trichocarpa*) by means of chlorophyll fluorescence imaging. In both types of signalling, PSII quantum yield of electron transport is reduced significantly. Two-dimensional imaging analysis of the fluorescence signal manifests the yield reduction which spreads via the veins through the leaves. The different types of signal propagation were shown by microelectrode measurements in leaf-vein phloem. Moreover, gas exchange measurements revealed that the depolarising signals travel distances across the stem to neighbouring leaves where the net CO₂ uptake rate is temporarily depressed towards compensation. We therefore conclude that electrical signals significantly affect the photosynthetic performance of poplar leaves.

P0440. The selfish module: the emergence of complexity in plant form and function

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Although individual plants exhibit a wide range of complex behaviour in response to environmental stimuli, they appear to do so without any identifiable centres of organisation. One way of explaining this is to suppose that plants are modular organisms, with whole-plant organization and behaviour emerging solely from the interactions of individual modules. Behaviour at the population and community levels of organization might also emerge from this same source. To test these ideas, we created a cellular automaton model of plant growth: a two-dimensional section depicting the plant in its above- and below-ground environments. The whole plant is represented by branching structures made up from identical 'modules'. From real experiments done on these virtual plants we have reproduced a wide range of whole-plant-, population- and community-level behaviour. All of these properties emerged successfully from a ruleset acting only at the level of the individual module.

P0441. Influence of salt stress on biosynthesis and emission of BVOC from grey poplar (*Populus x canescens*)

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Many plants produce isoprene via the plastidic isoprenoid pathway in large quantities. Light and temperature are known as the most important factors triggering isoprene emission at short and long term scales, while about the influence of other environmental factors less information is present. The actual study on poplar therefore aimed to elucidate if salt stress, an environmental stress factor with globally increasing importance, influences isoprene biosynthesis as well as the emission of other volatile organic compounds as acetaldehyde.

The presentation summarizes our actual knowledge on gene expression and biochemistry of isoprenoid genes, as well as photosynthetic gas exchange and VOC emission rates. The

observation that biosynthesis and emission of isoprene and oxygenated VOC were abided under salt stress even when photosynthesis was significantly lowered and leaves started to become damaged gave rise to further questions concerning the physiological function of this behaviour.

P0442. Shade-avoidance response in tomato, *Lycopersicon esculentum* Mill.

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Plants have evolved a number of photosensory systems that allow them to sense neighbors and compete for light or to decrease negative consequences of poor light under the canopy. The signal role of light in the adaptive response triggering was studied in tomato genotypes representing various morphological biotypes and various coenotic strategies. An early neighbor-sensing resulted in the predicted shade-avoidance response due to the unique mechanisms based on the "outstripping reality reflection" (pre-adaptation). There was a significant coenotic effect in the indeterminate growth forms as compared to determinate and dwarf ones. Two-phase response mode in the ontogenetic changes of the successive leaf blade area and specific leaf area was observed. Accelerated early growth was followed by the reduction of dry matter accumulation due to the lack of substrate light for photosynthesis. Later, transition to flowering and changes in the source-sink relations stimulated plant photosynthetic activity again.

P0443. Ethylene is involved in vascular cavity formation in pea (*Pisum sativum*) primary roots.

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A large cavity usually forms in the vascular cylinders of pea primary roots in response to sudden flooding at 25 C. This is thought to be a form of aerenchyma. Ethylene has been shown to mediate inducible aerenchyma in maize, therefore the role of ethylene in the formation of cavities in pea roots was examined. Pea seeds were germinated and grown in suitable containers for 4 d at 25 C in the dark in slightly moist vermiculite. Containers were then exposed to various concentrations of ethylene for 1 d or were flooded and endogenous ethylene measured periodically by GC. Also pea seedlings grown for 4 d in 2 l beakers in slightly moist vermiculite were flooded with solutions containing ethylene inhibitors or EGTA. Free-hand sections of the roots were taken. Ethylene caused cavities to form in a dose-dependent manner. Flooding increased ethylene amounts in the air space above the medium surface. Inhibitors of ethylene and EGTA suppressed cavity formation in flooded roots.

P0444. Winter-deciduous woody plants living under Mediterranean climate: phenology and internal nutrient cycling in *Amelanchier ovalis* Medicus

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The goals of this work were 1) to investigate whether *Amelanchier ovalis*, a winter-deciduous shrub of Mediterranean and sub-Mediterranean regions, has nutritional and phenological traits in common with temperate-deciduous phanerophytes; and 2) to determine the constraints of the Mediterranean environmental conditions on these traits. Phenology and leaf NPK in the crown were monitored monthly over two years. Leaf survivorship and nutrient resorption from senescing leaves were used to infer retention times of nutrients. Bud-burst occurred earlier than it does in temperate-deciduous phanerophytes. Most of the vegetative and reproductive growth was carried out in spring, although some phenological development occurred during the summer drought, chiefly fruit growth. Leaf shedding was very gradual, which might be due to water shortages in summer. Nutrient retention times were short compared to temperate-deciduous plants and co-occurring Mediterranean evergreens. *A. ovalis* showed phenological development appropriate for Mediterranean climates, although its limited ability to retain nutrients likely restricts the types of sites it can occupy.

P0445. Systematics and Pollination Biology of *Ophrys* (Orchidaceae)D. S. Devey^{1,2}, R. M. Bateman¹, M. F. Fay³;¹Natural History Museum, London, United Kingdom, ²University of Reading, Reading, United Kingdom, ³Royal Botanic Gardens Kew, London, United Kingdom.

Ophrys, a charismatic terrestrial insect-mimicking orchid genus with a mainly Mediterranean distribution, has long divided systematists as to the number of truly biological species. Use of standard morphometric techniques alone has been insufficient to distinguish the 215 currently named species. Here we utilise amplified fragment length polymorphism (AFLP) markers in conjunction with classical morphometrics and ordination analyses to further elucidate evolutionary relationships. A total of 75 putative species representing 23 of the 26 *Ophrys* groups recognised by Delforge, were collected throughout Europe and phylogenetic reconstructions based on UPGMA and Neighbor-Joining techniques were obtained. The preliminary results allow us to confirm the polyphyletic status of Section *Ophrys* and the monophyly of Section *Pseudophrys* (*sensu* Bateman). Additionally, we can establish that section *Ophrys* is not derived from section *Pseudophrys* (*sensu* Delforge), and we discuss convergent evolution of pollinator driven floral morphologies within Delforge's *O. fuciflora* s.l. and *O. bornmuelleri* groups.

P0446. Do pollinators really act as isolating factors in the sexually deceptive orchid *Ophrys*?P. M. Schlüter^{1,2}, P. M. Ruas^{1,3}, C. F. Ruas^{1,3}, G. Kohl¹, T. F. Stuessy¹, H. F. Paulus²;¹Institute of Botany, Vienna, Austria, ²Institute of Zoology, Vienna, Austria, ³Departamento de Biologia Geral, Londrina, Brazil.

The Mediterranean genus *Ophrys* (Orchidaceae) is pollinated by sexual deception. High pollination specificity by male insects is attained by mimicry of the pollinator female's sex pheromone. This potentially enables pollinators to serve as isolating factors and therefore variation in odour bouquets may cause a pollinator shift which may in turn lead to sympatric speciation. The island of Crete is remarkable for its diversity of *Ophrys fusca*-like taxa that are pollinated by different species of *Andrena* (Hymenoptera: Andrenidae) and several orchid taxa have been described based upon different pollinator specificities. Thus, we have used molecular markers to infer the phylogenetic history of east Mediterranean *O. fusca* s.l. taxa so as to test the hypothesis that pollinator specificity may serve to delimit evolutionary units. Genetic data, with an emphasis on AFLP fingerprint data, will be presented and their implications discussed.

P0447. Observations on Pollination Ecology of Seasonal Ornamental PlantsJ. A. Tidke, R. O. Dharamkar;
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This paper discusses the pollen production, pollen viability, visitors census and behavior of insect visitors in *Papaver rhoeas* Linn., *Antirrhinum majus* Linn. and *Salvia farinacea* Benth. The observations on different aspects involved in the studies of pollination ecology were undertaken during 2001 to 2004. The insect visitors were observed for their activity and behavior during the visits. Flowering poppies attract large number of insect visitors especially honeybees for collection of the pollen grains. In *A. majus* and *S. farinacea* also bees visit the flowers to collect the pollen grains and the butterflies for nectar. The insect visitors of these plants were bees; mostly *A. florea*, *A. dorsata* and *A. cerana indica*. Butterflies *Catopsilia pyranthe*, *Eurema hecabe*, *Danaus chrysippus*, *Danaus lyneus* visit the flowers frequently. The average pollen production per flower was found to be in the range of 535440±1087800 in *P. rhoeas*, 100800±253680 in *A. majus* and 6160±14280 in *S. farinacea*. The viability percentage was found to be in the range of 91.09 -94.17% in *P. rhoeas*, 98.29 -99.83% in *A. majus* and 94.09 - 96.96% in *S. farinacea*.

P0448. Pollination system and seed propagation of rare species and natural hybrids *Cypripedium* L. (Orchidaceae).E. V. Andronova;
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The experiences for artificial pollination and hybridization and the studying of seed and seedlings developmental biology in introduced plants (*C. macranthon* Sw., *C. calceolus* L., *C. shanxiense* S.F. Chen and natural hybrids) were carrying out

during ten years. Seed production in case of artificial self-pollination reduced. Pollen abnormality and a low seed set is characteristic feature of *C. x ventricosum*. Not numerous seedlings were unviable. Abundant seeds formed in crossing *C. x ventricosum* with *C. macranthon* and *C. calceolus* (back crossing). They germinated fast, seedlings develop normal, and it is similar with protocorm development in *C. macranthon* and *C. calceolus* and in interspecific hybrid (F1). A few characteristic features of reproductive structures in *C. shanxiense*, *C. macranthon*, and *C. calceolus* indicate that autogamy may take place in Far - Eastern population. Analysis of results for natural and artificial systems of pollination and crossing and a reason of high level of variability was carried out.

P0449. Structure of the labellum of *Ophrys bombyliflora* (Orchidaceae)A. Francisco, L. Ascensão;
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Ophrys orchids developed a highly specialized pollination system by sexual deceit. Flowers attract hymenoptera insect males by mimicking the females' appearance (shape, pilosity, colour pattern) and even the odour of their sexual pheromones. The morphology and anatomy of the labellum of *O. bombyliflora* are studied. The indumentum of its adaxial surface is composed by various types of epidermal cells whose distribution pattern changes according to the lip region. The basal part of the labellum is glabrous while the lateral lobes are villous. On the median region, the short-pointed unicellular trichomes of the speculum become smaller and scarcer near the lip margins. Conversely, the trichomes become longer and denser towards the distal part of the apical lip, where they acquire a moniliform shape. In this region, the labellum is thinner and bends abruptly downwards, giving rise to a reflexus appendix that is almost glabrous. The presence, on its abaxial surface, of epidermal papillae with secretory features and parenchyma cells with starch-rich plastids suggests that the appendix may be the osmophore, i.e. the site of synthesis and elimination of volatiles.

P0450. Lack of floral rewards promotes outcrossing: nectar supplementation experiments with *Disa pulchra* (Orchidaceae)J. Jersakova^{1,2}, S. D. Johnson²;
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The absence of nectar in many orchids has been hypothesized to cause pollinators to visit fewer flowers on a plant, and thus reduce self-pollination. This could increase fitness by reducing inbreeding depression and promoting pollen export. Previous investigations of this hypothesis have been conducted on bee-pollinated orchids. We studied the effects of adding sucrose solution to the spurs of the non-rewarding long-proboscid fly-pollinated orchid *Disa pulchra*. Addition of nectar significantly increased the number of flowers probed by flies (2.6 times), the time spent on a flower (4.5 times), the number of pollinia removed per inflorescence (4.8 times) and the probability of removed pollen being deposited on self-stigmas (3 times). Distances flown by pollinators on departure from experimental inflorescences (a possible measure of the likelihood of bi-parental inbreeding) were not significantly influenced by the presence of rewards. These findings provide strong empirical support for the idea that floral deception serves to minimize self-pollination and maximize pollen export.

P0450a. Pollination of *Brassia antherotes* Rchb.f. (Orchidaceae) in a Tropical Humid Forest at the Research Center La Montaña del Ocaso (Quindío-Colombia)N. H. Ospina, M. Diazgranados;
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We studied the reproductive phenology of *Brassia antherotes* in a tropical humid forest in the Colombian Central Andes. For the genus *Brassia* botanist have reported pollination by deceit, although contradictory evidences exist. The population analyzed is about 60 adult plants with a vertical distribution between 0.5 and 30m. The anthesis happens at 4:30h, and between 11-16h the flowers have a strong fragrance. Each flower stays opened along 7 days and the inflorescence along 15 days. All the population blossoms synchronically during 6 weeks with a peak of flowering between the 4th and 5th week, with 70% and 58.6% opened flowers, respectively. We found highly significant differences between the number of opened flowers (OF) and numbers of lost anthers (LA)

and closed stigmas (CS) during the 4th week (OF 285, LA 167, CS 14, 0.05, P 0.0001). In contrast, at the 5th week there were minor significant differences (OF 346, LA 344, CS 88, 0.05, P 0.0171). This displacement of the phenophases coincides with other studies about deceit and mimicry syndromes. Finally, we found at the end of flowering 80 closed stigmas that formed 18 fruits, and after 3 months they began dehiscence.

P0451. Anodic Asymmetry of the Secondary Leaves of *Croton variegatus* 'Banana'

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Secondary leaves of *Croton variegatus* 'Banana' form off primary leaf blades and have no petiole. All 260 secondary leaves were on the anodic side of the primary leaf blade whether the genetic spiral was clockwise (39) or counterclockwise (42). In all 12 cases of secondary branches changing genetic spiral direction the secondary leaves also changed direction to remain anodic. Three other cases were also examined. The larger of two axillary buds in *Acalypha virginica* (three-seeded mercury) in all of 119 leaves examined were anodic regardless of the direction of the genetic spiral. Also, the midrib in all 160 leaves of *Syngonium podophyllum* examined were curved in the anodic direction as were the coiling in 32 of 35 embryonic leaves. The data suggests that any phyllotactic model has to include a physical connection between leaves and this connection has to have an asymmetric component. It seems only the primary vein model of Esau and Sterling has this feature.

P0452. Developmental stability in leaves of *Plantago major* L. (Plantaginaceae)

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During June of 2004, leaves of common plantain (*Plantago major* L., Plantaginaceae), were sampled from a polluted site (Karaburma) and from a reference area (Zemun/Gornji Grad). Developmental stability of leaf traits (leaf width and vein distances within a leaf) is estimated using four indices of fluctuating asymmetry (FA_4 , FA_5 , FA_{10} and i^2) and by directional asymmetry (DA). Plants from the polluted site had sharply increased levels of FAs compared with plants from the control area. Comparison of the i^2 values between the two sites show that plants from the polluted area had significantly higher level of FA, for vein distances within a leaf, compared to those from the reference site ($F=6.167$, $p<0.01$). Results on directional asymmetry show significantly higher value of directional asymmetry for vein distances within a leaf in polluted area than in the reference one ($F=49.014$, $p<0.01$). Obtained data from fluctuation asymmetry analysis are in accordance with the directional asymmetry estimates. Reporting higher values of DA on the environmentally stressed site, our study corroborates the potential of DA as an indicator of developmental instability.

P0453. Apple genes that control the switch from vegetative to reproductive phase

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Studies on the mechanism of flower bud formation in the crops are very important because the process of flower bud formation is linked directly to the production and the quality of the crops. In tree fruits, the mechanism of flowering has remained to be cleared. Thus, we have studied the apple (*Malus x domestica*) genes putatively involved in the regulation of the switch from vegetative to reproductive phase to clear the function of the genes and to apply them to the reduction of the juvenile phase in fruit trees such as apple. Expression and function analysis of *MdAP1* (*APETALA1* ortholog) and *MdTFL1* (*TERMINAL FLOWER1* ortholog) demonstrated that *MdAP1* accelerates the flowering and that *MdTFL1* represses the flowering in transgenic Arabidopsis ectopically expressing *MdAP1* or *MdTFL1*. *MdAP1* starts to be expressed 2-3 month after flower bud differentiation in apple, suggesting that it is not involved directly in flower induction. On the other hand, *MdTFL1* is expressed transiently before and after flower bud differentiation in the SAM. Recent transgenic works in apple suggest that *MdTFL1* functions as one of the regulators of the switch from vegetative to reproductive phase.

P0454. Functional Characterization of Pollen Specific Gene *zm401* from *Zea mays*

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By differential screening of pollen cDNA library of *Zea mays*, we obtained nine clones. The full length of one of these clones, designed *zm401*, was obtained by 5' RACE. Molecular analysis showed that *zm401* is specifically expressed in pollens. This suggests that *zm401* likely functions in pollen growth and/or development. *zm401* was ectopically expressed in tobacco and overexpressed in maize under the control of a pollen specific promoter *zm13*. Interestingly, all transgenic tobacco and maize plants showed various levels of sterility, ranging from abortive flower development to male sterility. Further analyses on anther development indicated multiple abnormalities of anther development at late stages. These abnormalities include lagged degradation of the tapetum and connective tissue, failed deposition of fibrous bands in endothelial cells, and aborted pollen grain development. These strongly suggest that *zm401* has essential roles in anther development and its ability to perturb the anther development in heterogeneous dicotyledonous tobacco and maize indicates that *zm401* functions in a molecular mechanism possibly conserved in both monocots and dicots.

P0455. Determination of the division site in brown algal cells

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Different and sometimes contradictory mechanisms have been described for brown algal cytokinesis. Although brown algal cells lack cortical microtubules (MTs) and thus preprophase MT band as well as phragmoplast or phycoplast, both cytoskeletal elements, i.e. MTs and actin filaments (AFs) participate in the formation and fixation of the cytokinetic diaphragm. Treatment with anti-MT or anti-actin drugs inhibits cytokinesis. In the present work dividing cells of *Sphaecelaria rigidula* were monitored during recovery after treatment with nocodazole and cytochalasin B that impair MT dynamics and cause AF fragmentation respectively. In both cases a ring of wall material displaying numerous plasmodesmata was formed in the expected cytokinetic plane, even in cases the daughter nuclei do not occupy the correct positions as well as in cases in which the forming cytokinetic diaphragm was displaced by large vacuoles. These data suggest that the division site in brown algal cells is determined before cytokinesis or that in this site some mechanism(s) function, favouring wall deposition in the absence of both MTs and AFs.

P0456. Novel mutants for the study of hydrotropism in Arabidopsis roots

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We found that Arabidopsis roots easily displayed hydrotropism in response to moisture gradient. Using this system, we isolated Arabidopsis mutants of which root hydrotropism was impaired or altered, and named *root hydrotropism* (*rhy*). Roots of *rhy1* and *rhy4* did not show hydrotropism but normally exhibited gravitropism, phototropism and waving response. Roots of *rhy2*, *rhy3* and *rhy5* showed reduced hydrotropism together with altered response in gravitropism, phototropism and/or waving response. Elongation growth of *rhy* mutant roots, except for *rhy3*, was similar to that of the wild type. However, roots of *rhy4* and *rhy5* showed a reduced elongation growth when waving response and hydrotropic response were, respectively, induced. Thus, we obtained unique hydrotropism mutants of Arabidopsis, which will enable us to dissect molecular mechanisms for hydrotropism and its interaction with other growth movement in roots.

P0457. The theory of critical periods in flowering plants ontogenesis.

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The theory of critical periods in plant ontogenesis has been elaborated from studies of integral morphogenetic processes on different organization levels. Periodization of various reproductive structures development has been worked out from data on morphogenesis using systemic & complex approaches. Critical

phases, stages & periods have been revealed, e.g. the stage of embryo autonomy in angiosperms by means of culture in vitro. The concepts of "critical period" & "critical mass" in relation to embryonal structure periodization are discussed. Also addressed are the question of allometry & the significance of morphogenetic fields & rhythms of cell division for revealing critical periods and the management of ontogenesis. In development of anthers and ovules has discovered 3 common critical periods: premeiotic, meiotic & postmeiotic. Embryo development in angiosperms is characterized by 2 phases (proembryonal & embryonal) and 5 critical periods (zygote, proembryo, globular, heart- & torpedo-shaped, mature embryo). Switching of the developmental program at certain critical periods is caused probably by the functioning of stem cells in various organs.

P0458. Effect of Electrogenic Flower Induction on Expression of FLORICAULA/LEAFY Orthologue in *Chenopodium rubrum* L.

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Signal transduction during photoperiodic flower induction includes a network of both molecular and physiological changes. The transcription factor *LEAFY* (*LFY*) of *Arabidopsis* is homologue to *FLORICAULA* (*FLO*) of *Antirrhinum* and is assumed to be involved in flower initiation. We have identified the *FLO/LFY* orthologue in *C. rubrum* (*CrFL*). The expression pattern of *CrFL* at the shoot apex is modulated by photoperiodic flower induction. We succeeded to substitute the inductive photoperiod by a specific pulse-pattern of applied electric current. In order to analyse the role of putative electric signals in the signalling network, we followed expression of *CrFL* under electrogenic flower induction using real time PCR. We could show that the expression pattern of *CrFL* changes under electrogenic flower induction. Therefore, electric signals seem to lead to flowering over similar signalling pathways as the flower inducing photoperiod. We assume that electric signals might indeed play a role in signal transduction in flower initiation.

P0459. Concept of plant stem cells (smbryological view)

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Analysis of data on embryology of flowering plants and stem cells (Barlow 1978, 1997) allowed firstly manifest that the formation stem cells occurs in all organs (flower, stem, leaf, root), at all stages of life cycle (sporophyte, gametophyte). Their functioning first of all depends on their localization & destination. Zygote is a unique stem cell - "grandparent" of not only apical root & shoot meristem, but also stem cells of other orders (hypophyseal & epiphyseal in sexual embryo, cambium, "dormant" meristem in leaves, apical part of primordia of ovule & anther & also the initial cells of somatic embryos of different origin). The main features of plant stem cells are given: 1) toti- or pluripotency (ability to form tissues, organs & new individual); 2) relative dormancy; 3) two types of division: symmetrical divisions cause self-maintaining pool of stem cells, asymmetrical - formation "niches" & initial cells of different tissues; 4) the pulsating & multistage character of formation & the capacity to switching over the developmental program. The differences of plant stem cells & their functioning in the system of "reserves & refusals" are discussed.

P0460. Jasmonic acid and wounding promotes apospory in fern leaf culture

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The effect of jasmonic acid (JA) and wounding on apospory - development of gametophytes from sporophytic tissue without meiosis - in leaf culture of the fern *Platyserium bifurcatum* (Cav.) C. Chr. was investigated.

Wounded and intact leaves were placed flat with the abaxial side on MS growth media with different concentrations of JA (0; 0.1; 1; 10; 100 µM) and 0.01 % sucrose. JA promoted development of aposporous gametophytes in wounded leaves and had no effect on the intact leaves. On the medium with 0.1-10 µM JA the number

of aposporous gametophytes was higher compared to the medium without JA. There was no influence on the number of leaves with apospory. Aposporous gametophytes developed on both sides of the leaves. Beside gametophytes, rhizoids and adventitious shoots also developed. Our findings suggest that JA in combination with wounding may be involved in apospory development.

P0461. Expression and localization of *Arabidopsis* synaptotagmins

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Synaptotagmins are known Ca²⁺ dependent triggers of exocytosis and endocytosis in animals with nervous system. Sequence analysis of various animal and plant genomes revealed the presence of synaptotagmin genes in all animals and land plants, but there is no evidence of synaptotagmin genes in unicellular organisms or those with simple forms of multicellularity. Synaptotagmins have a common structure, a N-terminal transmembrane sequence is followed by a linker and two distinct C2 domains, C2A and C2B. These two domains are known Ca²⁺ binding domains, but there is nothing more known about the function of the proteins in plants. To get more information about the 6 members of the *Arabidopsis* synaptotagmin gene family, we are investigating expression patterns by promotor-GUS fusions and cellular localizations by GFP-fusions as well as antibody labelings. Preliminary data suggest that the members of the synaptotagmin gene family are differently expressed, but show similar cellular localization.

P0462. Root - Shoot - Signaling in *Chenopodium rubrum* L. as Studied by ¹⁵O Labeled Water Uptake

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It has been demonstrated with *C. rubrum* that the different organ systems are transmitting surface action potentials which might be the basis for systemic signal transduction. Shoot tip respectively root generated action potentials travel along the stem axis. Shoot tip generated action potentials arriving at the basis can be reflected and travel upwards. The radioactive labeling technique was established at the NIRS in Inage, Japan. About 2GBq of ¹⁵O-labeled Hoagland's solution was supplied to the plant root or cut stem in a phytotron at 25°C with 45 % of relative humidity and continuous light. By cutting the shoot apical bud and the apices of main side branches the uptake of ¹⁵O labeled water was inhibited in plants with intact roots but not in plants with roots cut. Because of the short half-life of ¹⁵O (2 min), experiments could be repeated in hourly intervals. Cutting the apex probably limits root water uptake via a hydraulic-electrochemical signal. The results are discussed with respect to the significance of a continuous communication between the root system and the shoot apical meristem(s) in the adaptation of plants to their environment.

P0463. Phosphoinositides in endocytic pathways: Can the phosphate position change the endosomal targeting?

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Phosphoinositide-based signalling is thought to be a part of plant responses to various biotic or abiotic stresses. Upon a stress, plant cells can produce various phospholipids that play role in cell signalling by usage of protein with specific lipid-binding sites, like FYVE, PH or PX. We demonstrate that GFP-FYVE fusion protein is recruited by endosomes via interaction with PI3P being abundant in early/recycling endosomes which are highly motile and serve as a sorting stations for endocytic cargo. On the other hand, *Arabidopsis* TRITHORAX1 is a chromatin remodelling protein that binds specifically PI5P. Surprisingly, TRITHORAX1 localize besides nuclei also to the plasma membrane and to non-motile endosome-like compartments. Myotubularin phosphatases can change the position of the phosphate on the phosphoinositides

to generate PI5P from PI3P. This conformational change is predicted to recruit ATX1 to the endosomal membrane via changing of its targeting. *Arabidopsis* genome encodes two myotubularin homologues allowing us to dissect the endocytic pathways based on PI3P and PI5P phosphoinositides.

P0464. Is the control of the cambial cells readjustment based on the negative feedback?

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Intrusive growth of cambial initials has a role in readjustment of cells in the vascular cambium as it affects changes in cell orientation, cell tip inclination and mutual arrangement of fusiform initials as also the changes in shape and / or inclination of rays. Based on experimental data, it was assumed that cambial cells readjustment is strictly connected with changes in the patterns of mechanical stresses. The existing hypotheses explain the phenomenon of cambial cells readjustment as a linear relationship e.g with auxin flow. Our studies propose existence of a system, based on the negative feedback, for the control of cambial cells readjustment. This system includes relations between all cell events and the tension, compression and shear stresses, showing the plurality of environmental modifiers of the process. The proposed hypothesis considers the cambium as an early-warning, self-regulating system which enables the tree to adapt its structure to the constantly changing conditions of the internal and external environments. This regulating system relaxes the threshold stresses smoothly before they may become a threat to the plant.

P0465. The guild of hummingbird-pollinated species in an Atlantic forest remnant, northeastern Brazil

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Hummingbirds are the major vertebrate pollinators of Neotropical plants and pollination by these birds is found in many botanical families. Studies on pollination guilds are an efficient way to understand ecological processes in natural ecosystems. In a two-year study of the ornithophilous guild of an Atlantic forest remnant, in northeastern Brazil, 29 species from 17 families and 25 genera were recorded. Species of Bromeliaceae are predominant (27.6%). Most species of this guild are epiphytes (64.3%), and the majority showed reddish/pink flowers (55.2%) of the tube type (79.3%). Nectar sugar concentration and volume ranged from 10.2-46.8% and 2.5-97.4 µl, respectively. A high number of species (91.7%) bloomed in the rainy season. Eight hummingbird species were recorded, being the Phaethornithinae, *Glaucois hirsuta* and *Phaethornis ruber*, and the Trochilinae, *Amazilia fimbriata* the main pollinators. Among them, *Glaucois hirsuta* behaved as the dominant species. In comparison with ornithophilous guilds of other Neotropical areas, this forest remnant revealed richness of species and interactions.

P0466. Breeding System of *Campanula lusitanica* L. (Campanulaceae)

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Phenology, floral characteristics, pollinator spectrum and breeding system of *Campanula lusitanica* s.s. were studied in two population from Southern Spain. Flowering extends from early May to mid-June. Flowers are protandrous and male phase lasted 2-3 days. Total floral longevity ranged from 5 to 6 days. Diptera and Himenoptera were the main visitors of *C. lusitanica* flowers, and fruit set in natural populations was very high ranging from 88.6 to 93.3 %. Results of geitonogamous pollinations showed that this species is self-incompatible and observations of pollen tube growth indicate that the incompatibility system is gametophytic. Hand pollinations between *Campanula lusitanica* s.s with both *C. lusitanica* var. *broussonetiana* and *C. lusitanica* subsp. *specularioides* results in absence of fruiting. These preliminary

results suggest the existence of hybridation barriers between the taxa included in *Campanula lusitanica* group.

P0467. The asymmetric flower of *Vigna caracalla* (Fabaceae: Papilionoideae): mechanical aspects of its pollination mechanism.

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The flower of *Vigna caracalla* (L.) Verdc. has a pronounced asymmetry and 5 revolutions of the keel. Instead of its complexity, it resulted functional enough, with a brush mechanism activated by pollinators capable of execute a considerable strength (*Bombus morio*, *Xylocopa eximia* and *Centris bicolor*). To reach the nectar chamber, the bees have to lower the left wing with their legs, and this effort results in the emergency of the stigma and ca. half turn of the stylar brush charged with pollen. A force of 0.053 ± 0.006 N is needed to trigger the flower. The distance from the centre of rotation was 14.6 ± 0.3 mm, resulting a moment of rotation of $7.3 \times 10^{-4} \pm 1.03 \times 10^{-4}$ N m. Calculating with the weight of *B. morio* (36 mg), an lever arm of 21 cm would be necessary if the insect tried to open the flower by weight only. *Apis mellifera* and some species of Lepidoptera were unable to operate the pollination mechanism of *V. caracalla*.

P0468. The feature correlations in mutant plants of *Verbena canadensis* Britt.

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To induce variation of decorative features for use in selection, seeds of *V. canadensis* were treated by diethyl sulfate. The morphologies and colours of the resulting M1-M6 flowers have been analysed. The nine flower colours ranged from pale rose to purple including lilac colour and differed in intensity, a bluish shade and the presence of a white eye. The flowers differed in sizes, shapes, the tube dimensions and the limb diameter, the features found to be correlated.

All F1 hybrids from the reciprocal crosses plants with large pale rose and small violet-purple flowers had the rosy lavender large flowers. The flowers of different colours were pollinated by different insects, the control magenta-rose flowers by butterflies, the violet and lilac-coloured ones by bumble bees (*Bombus*), and the pale rose and lavender hybrids by hawk moths (*Sphingidae*) even in the cloudy weather. The model population of the mutants showed apparent linkage of the flower colour to the other traits and to the pollinators.

P0469. Seasonality in ornithophilous flora and hummingbirds in a montane Atlantic forest of Southeastern Brazil.

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Studies of plant-pollinator interactions in plant communities can contribute to an understanding of evolutionary and ecological questions. A community of ornithophilous plants was studied in a montane forest in the Parque Nacional do Itatiaia, with the aim to relate floral morphology and biology to the characteristics of their hummingbird pollinators. In a two-year study 30 plant species were recorded, being epiphytic/herbaceous species of Bromeliaceae, Gesneriaceae and Acanthaceae the most frequent. Effective corolla length was short (<2 cm) in 5 species, medium (2-4 cm) in 21 species and long (>4 cm) in 4 species. Flowers with medium and long corollas predominated during the dry period (winter), and short flowers predominated during the rainy period. Accompanying the seasonal variation in flower size, visits of two long-billed hummingbird species predominated during the winter, and visits from a short-billed species were frequent in the wet period. These results indicate that the seasonality of flowering in species with different flower sizes may be an adaptive response to the different availabilities of hummingbird pollinators during the year.

P0470. Rushes are different

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We analysed the breeding system of *Juncus atratus* by a combination of genetic, phenological and modelling approaches. Unlike other wind-pollinated taxa in the genus *Juncus* low pollen to ovule ratios are found which leads to the assumption of high selfing rates.

However, microsatellite analysis in *Juncus atratus* revealed a contrasting view. Outcrossing rates obtained from Fis contradict an autogamous breeding system.

Two possible explanations can be specified: 1) Selection for heterozygosity. 2) Increased outcrossing rate despite low P/O ratios.

Many members of the genus show a very peculiar flower phenology, best described as synchronous pulsed flowering. The flowers of a whole population simultaneously open for a few days in a more or less even rhythm.

This phenomenon may lead to a higher outcrossing rate. This can be supported by a model using empirical data and could explain in part the discrepancy between the autogamous appearance of the flowering syndrome and the genetic findings.

A genetic analysis of single seeds will provide a direct measure for the amount of outbreeding in *Juncus atratus* and can show the effectiveness of this special flower phenology.

P0471. Floral resource for birds in the canopy: *Spirotheca passifloroides* (Bombacaceae) and its visitors in the rainforest of Southeastern Brazil

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Birds are a very important component for plant reproduction in tropical ecosystems, being hummingbirds the most important vertebrate pollinators in the neotropics. In a two-year study on floral resource availability for birds were recorded 56 species visited by hummingbirds, from these only four species were also visited by other birds. Most species are epiphytes, few are shrubs, lianas or trees; the majority occurs in the understory and flowers during the wet season. A few species occur in the canopy, such as *Spirotheca passifloroides* an up to 30m high tree, which flowers during the drier season. Individuals may open up to 180 flowers/day, which are red and tubular (40mm length x 11mm diameter). Accumulated nectar volume and concentration is on average 142µl and 6%, respectively. These big flowers were visited by 11 bird species, among hummingbirds, passerines and non-passerines. Although these bird species have different sizes and visiting behavior, most of them are pollinators of *S. passifloroides*. This Bombacaceae diverges from most species in the community and is an important canopy resource in a period of food scarcity.

P0472. Asymmetric flowers and phylogeny in *Senna* (Leguminosae, Cassiinae)

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Senna (Leguminosae) is a large and diverse genus with buzz-pollinated flowers and many species are characterized by asymmetrical (enantiostylous) flowers. Our maximum parsimony analyses based on the plastid regions *rps16*, *rpl16*, and *matK* (exon part) are aimed at testing the current classification of the genus and reconstructing the evolution of enantiostyly in *Senna*. Of the six existing sections, only sect. *Psilorhegma* is monophyletic. Sections *Chamaefistula*, *Peiranisia* (with prominent floral asymmetry in addition to mere enantiostyly) and *Senna* are polyphyletic. The monotypic sections *Astroites* and *Paradictyon* group with species of sections *Chamaefistula* and *Senna*, respectively. The phylogenetic analysis retrieved seven major clades. Two clades include all taxa with monosymmetric flowers. The five clades with enantiostylous flowers are each characterized by a distinctive variant of floral asymmetry, in which different floral parts are involved in addition to the gynoeceum. We will show current results of our morphological and developmental studies aimed at understanding the diversity of floral asymmetries and their evolution in *Senna*.

P0473. Identification of the ABC floral gene orthologs in *Viola pubescens*

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Most violets can produce both chasmogamous and cleistogamous flowers. Since the ABC model suggests A, B and C classes of genes play essential roles in the flower development, we are using PCR (polymerase chain reaction) to identify the ABC floral gene orthologs in a widespread violet, *Viola pubescens*, in hope of better understanding the molecular mechanism underlying this floral dimorphism. Primers were designed by aligning the known "ABC" orthologs in other plant species. Genomic DNA extracted from *V. pubescens* was used as the template. PCR products were cloned and sequenced. The sequences were then subjected to the nucleotide-nucleotide BLAST (basic local alignment search tool) to verify their identities. In this way, two A class orthologs, two B class orthologs and one C class ortholog were identified. The future work is to compare the expression of these ABC orthologs along the development of the two types of flowers and to study their regulation. Our research will improve the knowledge of the development of the two types of flowers in *Viola* from the molecular point of view.

P0474. Keel anatomy of two *Mucuna* species (Leguminosae, Faboideae) helps to understand the explosive opening of their flowers

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The exhibition of the reproductive organs in *Mucuna* happens through explosive flower opening. At the tip of the keel the petals are united to some extent and stiff, features which probably act as a support to bring about flower explosion after a visit. The anatomy of the apical region of the keel of *Mucuna japura* and *M. urens* was studied to verify how these features influence flower opening. In both species the inner mesophyll shows cell wall lignification, but secondary wall thickenings occur only in *M. japura*. In the non-lignified part of the inner epidermis of both species occur entwined secretory papillae, which keep the petals firmly united. Flowers of *M. japura* are more difficult to open than those of *M. urens*; the secondary wall thickening of the former could be associated to this characteristic. These features are related to the pollination biology of the *Mucuna* species, since *M. japura* is pollinated by a large passerine bird, *Cacicus haemorrhous* (Icteridae) and *M. urens* by a small bat, *Glossophaga soricina* (Glossophaginae), which have different foraging behaviors and apply different strength due to their sizes to trigger the explosive flower opening.

P0475. Nectar drinks for all, from lizards to doves: the vertebrate pollinators of a leguminous tree on an oceanic island off Brazil

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Oceanic islands provide remarkable instances of relationships between vertebrates and plants. At Fernando de Noronha Archipelago, off north-eastern Brazil, we found that all four native terrestrial vertebrates rely on the flowers of a leguminous tree. The lizard *Euprepis atlanticus*, the dove *Zenaida auriculata noronha*, and two perching birds, *Elaenia ridleyana* and *Vireo gracilirostris* visit and pollinate the tree *Erythrina velutina*. The trees' blooming period coincides with the dry season on the island, and the orange flowers are visited from dawn to sunset. The nectar is dilute (~10%) but produced all day long and copiously - an hourly secretion rate of up to 100 ml. A large tree may bear about 300 inflorescences, each with two to eight open flowers daily. As none of the visitors seem to depend energetically on nectar (the lizard eats anything edible, the dove is mostly a seed-eater, and the perching birds feed mostly on insects), we suggest that these visitors seek the flowers both for sugar and water intake. Additionally, we suggest that vertebrates' visits to flowers on small islands worldwide may serve a dual purpose: energy acquisition and water balance.

P0476. Legume leaf patterning genes

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The garden pea (*Pisum sativum*) is studied for comparative developmental genetics. In common with many other legumes, pea has architectural features that differ from other well-studied model species, including secondary inflorescences, bisymmetrical flowers and pinnate leaves. The ongoing identification of morphological mutants and their corresponding genes is beginning to reveal mechanisms that determine form in pea, particularly those that pattern the specialised compound leaf. *CRISPA* was identified recently as the orthologue of the Arabidopsis gene, *ASYMMETRICLEAVES1*. Mutant *crispa* leaves form ectopic stipules along the length of their petioles and this is associated with ectopic expression of the homeobox gene *PSKN2*. The *crispa* mutant phenotype resembles the winged petioles of some *Lathyrus* species eg. *L. annum*. *Pisum* and *Lathyrus* are closely related genera in the "vicioid" clade of papilionoid legumes. We are investigating shared and divergent morphological features in *Pisum* and *Lathyrus* by asking whether mutant pea phenotypes recapitulate *Lathyrus* species phenotypes and whether we can identify associated molecular markers.

P0477. Programmed Cell Death in the development of Tillandsia (Bromeliaceae) trichomes

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Tillands use their epidermal trichomes for absorbing water and mineral and organic nutrients from the atmosphere. The absorbing trichome in *Tillandsia* has a nail-like shape and is formed by an axis of 1-6 cells, connected to the internal tissues of the leaf, and to an external shield (or head). This last structure is mono-layered and formed by dead cells extending their surface over the epidermis. The solutions coming from the external environment go through the shield cells and follow a way through the axis cells of the trichome to reach the underlying mesophyll parenchyma.

The first studies on trichomes of *Tillandsia* and observations on the ontogeny of *Tillandsia* trichomes are of 1904, with Light Microscopy. Also because of some methodological difficulty, ultrastructural studies in *Tillandsia* are relatively few and not recent.

In this contribution we discuss the ultrastructure of the ontogeny of *Tillandsia* trichomes, with particular attention to the last stage in which the wing cells die at maturity. The ultrastructural features of these cells in the last stage of development are similar to those observed in other cases of Programmed Cell Death in plants.

P0478. Inflorescence morphology in Balanophoraceae: moving in a twilight zone.

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The pantropically distributed holo- and root-parasitic family Balanophoraceae has panicked, mostly spadiceous inflorescence types with a remarkable variety of different designs. Contrary to other authors, all inflorescences are initiated endogenously in subterranean globose or sometimes elongated tubers. Racemes (subfam. Mystropetaloidae, Helosidoideae and Balanophoroideae), double racemes (subfam. Dactylanthoideae and Lophophytoideae) and triple female racemes (subfam. Sarcophytoideae) can be found. In tribe Helosieae spadices are covered by a dense layer of hexagonal, umbrella like structures which could be identified as bracts (not secondary axes as discussed before), showing an up to now unknown and for leaves unusual developmental pathway of peltation. Balanophoroideae possess spadices without secondary branching (in tribe Langsdorffieae they are designed like Asteracean heads). Structures covering the spadix, formerly described as axes ("spadicelli"), turned out to be transformed bracts. But it is still unclear if these "spadicelli" originate from numerous individual bracts or few multiple splitted bracts.

P0479. Acquisition of root hemiparasitism and other life history traits in Santalales

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Based on molecular analyses, we examined the acquisition of hemiparasitism in Santalales, particularly Olacaceae s.l. Mapping

of life history characters on molecular cladograms suggests a single origin for an ancestral root hemiparasite that can be assumed to be one of two ecological types. The first is an under-shrub with relatively small fruits. This type, likely occurring in a humid forest, is reminiscent of the extant genus *Ptychopetalum*. The second type is a small tree with relatively large fruits. This type likely occurred in dryer areas (e.g. margins of humid forests and savannas) and is reminiscent of the genus *Ximena*. After the acquisition of root hemiparasitism in Olacaceae, additional trends include: the acquisition of accrescent structures around fruits, large tree habit (*Ongokea*), and hummingbird pollination (*Chaunochiton*). Other trends seen in Santalales include: five independent acquisitions of the mistletoe habit; several independent derivations of an herbaceous habit within Santalaceae; reduction in leaf size, venation and changes in phyllotaxy (Santalaceae and Loranthaceae); and pollination syndromes (Loranthaceae).

P0480. Taxonomic Revision of the genus Cunila (Lamiaceae) in North America and Central America

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Cunila D. Royen ex L., an American genus belonging in tribe Mentheae, includes approximately 20 species. Geographically has a disjunct distribution, one centre of diversity extends from the eastern United States to Panama and another group of species is located in south eastern Brazil, northern Argentina and Uruguay. *Cunila* is distinguished from other Lamiaceae by having five-teeth calyx and two exerted stamens. The genus, has never been the object of a complete revision, there is only a synopsis of the South American species. In this treatment, 7 species are recognized for the Northern region, the nomenclature is resolved and characters with systematic value are suggested. Examples of them are: growth habits, inflorescences, pedicel and peduncle length, calyx teeth symmetry and the way the trichomes are inserted in the calyx throat, as well as the morphology of the nutlets, these are apparently more useful for classification in the genus than pollen morphology, which is similar among species. Economic (medicinal and ornamental) importance and vernacular names are included for some species when known.

P0481. Force measurements in Salvia flowers

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Salvia flowers are characterised by the staminal lever mechanism. Insects and birds searching for nectar have to push back a barrier originating from the lower connective arms of the lever-like modified stamens. To test the hypothesis that weak insects are restricted by this barrier the forces needed to release the lever were measured by means of a special device. The forces of 25 species including bee pollinated and bird pollinated flowers do not show significant differences. Altogether they are rather low. They range from 0.10 mN ± 0.54 to 21.34 mN ± 21.88 showing high standard deviations in each species. No correlation was found with the age of the flowers, their size, the levers' proportions and the flower classes. These results indicate that there is no selection pressure on high or precise forces, that the lever movement instead has been selected to go easily and to allow a large range of insects access to nectar. It is assumed that the lever mechanism increases the precision of pollen transfer and possibly contributes to mechanical isolation among co-occurring species.

P0482. Study of anatomical structures, ontogeny and karyotypic Characteristics of Two Species of Salvia

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Salvia, the largest genus of Lamiaceae, includes about 900 species, widespread throughout the world. In the present study, anatomical structures of meristems, ontogeny of flower organs, microsporogenesis, megasporogenesis, embryogenesis, karyotypic specificities of two species of *Salvia* genus (*S. officinalis* L. and *S. sclarea* L.) were investigated and compared. The anatomical structure and ontogenical stages were similar in the two species. The inflorescences comprise verticillasters generally possessing 4-6 flowers with the bracts exceeding 20-30 corolla which can be purple or white in color with the upper lip strongly falcate. Anatomical studies showed that protruding meristems and indefinite inflorescences were similar in these two species. Study

of ontogeny of flower organs showed that sepals and petals appeared simultaneously and the stamens and the ovary appeared later. Ovules were anatropous and contained an integument, which was well developed. The embryo contained two small cotyledons and the seed contained a vast endosperm. Cytogenetic studies revealed the number of chromosomes in *S. officinalis* to be $2n=14$ and in *S. sclarea* it was $2n=22$.

P0483. The glandular structures of *Leonotis leonurus* during the course of flower ontogenesis

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Pursuing our studies in *Leonotis leonurus*, an African Lamiaceae species, the aim of this work is to characterize the secretory structures present on the flower during its ontogeny. In *L. leonurus*, the calyx abaxial surface presents a dense indumentum of mixed peltate and capitate trichomes, mostly covered by nonglandular hairs. On the corolla, capitate trichomes are rare while peltate are clustered on the abaxial side, mainly on the median region of the corolla tube, being sparse on the adaxial surface. On the stamens and carpels, capitate trichomes are absent. Peltate trichomes are abundant on the lower side of the anther, between the two lobes, and on the top of the ovary. A four-lobed asymmetrical nectary disc is observed on the base of the ovary. The nectar, sucrose-dominant, is released by modified stomata on the inner side of the nectary's largest lobe. On the inner basal region of the corolla tube five levels of well developed finger-like projections occur. These are columnar cells of a multi-layered glandular epidermis, which produce a lipophilic secretion. The function of these different types of glandular structures is discussed in relation to pollination.

P0484. Floral morphology of *Stachys* L. (Labiatae) and some aspects of its pollination.

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The floral architecture of most *Stachys* species is investigated. In general, its structure is rather monotonous (indistinctly bilabiate calyx and 2-lipped corolla, with stamens directed towards the posterior lip, rarely almost hidden in the tube). Some new diagnostic characters to separate *Stachys* sections and subsections are revealed, such as features of calyx, corolla and placement of stamens. Also, based on the character of long protruding stamens and some other features, the new subgenus *Menitskia* from West Himalayas is described. The structure of flowers is analysed in the context of pollination. The calyx is of little importance for pollination. The anterior corolla lip has a coloured "nectar-guides". In general, *Stachys* is a melittophilous genus. American species of the section *Calostachys* adapted to ornithophily. They have red corolla with long straight tube. Some Southern African species with long narrow corolla tube have long-proboscid flies as pollinators.

P0485. Phylogeny and Floral Evolution in Lamiaceae

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A phylogeny based on cpDNA sequences (ndhF and trnL-F) provides a basis to explore the patterns of floral evolution in Lamiaceae. Because sampling was designed to establish a phylogenetic framework for Lamiaceae as a whole, some large, well supported clades (e.g., Nepetoideae) are represented by only a few exemplars in the analysis. Mapping of morphological data onto the strict consensus tree reveals homoplasy in many floral characters that have traditionally been used to delimit lamiaceous taxa. For example, a gynobasic style evolved separately in Nepetoideae and Lamioideae, and a sunken style (intermediate between terminal and gynobasic) evolved independently in Prostantheroideae and Ajugoideae (probably several times in the latter). Dimidiate stamens evolved independently in Scutellaria, Anisomeles, and a subclade of Prostantheroideae. Other characters that evolved more than twice within Lamiaceae include actinomorphic corolla, persistent style, elongate anther connective, and confluence of anther locules.

P0486. Nectar production of three Lamiaceae species in relation to microclimatic conditions

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The nectar production of *Lamium maculatum*, *Lamiastrum galeobdolon* and *Ajuga reptans*, grown under different microclimatic habitat conditions (forest and experimental field), was evaluated by determining the total daily nectar quantity per flower, per plant and per square unit, diurnal dynamics of nectar secretion and nectar sugar concentration.

Nectar amount and sugar concentration varied among the studied species as a function of microclimatic parameters (air temperature, air humidity and evaporation) and corolla morphology. Higher nectar production and lower sugar concentration were recorded in *L. maculatum* and *L. galeobdolon* grown in the forest, while for *A. reptans* in the same habitat, the reverse results were obtained. Total daily nectar volume per plant was the highest in *L. maculatum* (30.1 ml/pl). The highest nectar yield per square unit was found in *L. maculatum* and the lowest in *A. reptans*. Regarding nectar secretion rate, *A. reptans* is the slowest producer, secreting less than 0.02 ml/h. With respect to secretion intensity, total daily nectar production per flower and the density of species population, the most melliferous species is *L. maculatum*.

P0487. Sharing without mixing? Quantitative analyses of pollen placement on *Apis mellifera* as a pollinator of *Salvia pratensis* and *Salvia nemorosa*.

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If sympatric *Salvia* species share the same pollinator, two scenarios are possible: (a) pollen is mixed and hybrids between the species may occur, or (b) pollen is not mixed because it is deposited on distinctly separated areas on the pollinators body. Our new data on pollen-placement of *Salvia pratensis* and *Salvia nemorosa* indicate that the pollen of these two species is mixed on the body of *Apis mellifera*, a pollinator shared by both *Salvia*-species. This finding is corroborated by the fact, that rare natural hybrids of these two species are reported by many authors. This raises the question why these two species are stable and do not merge to a single one. We list a number of mechanisms that might reduce (1) the frequency of hybrid formation and (2) the reproductive success of the hybrids. These considerations support the idea that selection against hybrids of *Salvia pratensis* and *Salvia nemorosa* is a complex multi-factorial process. An evaluation of the contribution of a single factor (as pollen placement) to the selection against hybrids requires a quantitative analysis of the processes as a whole.

P0488. The architecture of inflorescences in the Brazilian genus *Diplusodon* (Pohl) Lythraceae.

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In the monotelic type of inflorescence the axis terminates in a flower. In the polytelic lacks a terminal flower. In Lythraceae most of the genera are polytelic. In *Diplusodon*, one generally finds the polytelic pattern with flowers organized in a botry. In addition to the polytelic type described for *Diplusodon*, *D. ovatus* and *D. panniculatus* were found with monotelic inflorescence called thyrsoid, where the cymose partial inflorescences are triads, being able to continue to produce lateral flowers in the axils of prophylls and form a dichasium. It is considered that the polytelic type of inflorescence has been derived from the monotelic type, by loss of the terminal flower and specialization of the paraclades. In *Diplusodon* there are examples that correspond to the transition between the two types. One can see also in the two species that in their monotelic thyrsoids, the cymes that precede the terminal flower are reduced to botyroids, suggesting a transition for the polytelic pattern. The inflorescences in *Diplusodon* can be organized according to the evolutionary hypothesis for the inflorescences from the monotelic, toward the polytelic and reduction of flowers.

P0489. Radiation and evolution of the large woody genus *Gutteria* (Annonaceae)

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The Neotropical genus *Gutteria* is the largest within the family of Annonaceae and one of the largest woody plant genera in the Neotropics, comprising ~260 species. In the last revision (1939) division in 30 sections was based on few macromorphological characters. Up to now *Gutteria* is the only major genus of Neotropical Annonaceae that awaits revision. Because of its uniformity in many morphological characters it is very difficult to understand the systematics and evolution of the genus. In this study the position of *Gutteria* within the family and the relationships with three closely related smaller genera, *Gutteriopsis*, *Gutteriella*, and *Heteropetalum*, were investigated. To this end a parsimony analysis was performed using plastid sequence data. Although the position of *Gutteria* within the family has not been resolved fully, it is clear that *Gutteria* is sitting at the end of a long node, but divergence levels among the species are low. Furthermore, *Gutteria* forms a well supported monophyletic group, but *Gutteriopsis* seems to be paraphyletic with respect to *Gutteria* as a whole. It is also clear that the described sections can no longer be upheld.

P0490. Evolutionary Trends in Ovule Development and Organization

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Ontogenetic variations in ovule expression might have occurred during its evolution. At first the nucellus was connected to integument(s) by a stalk, and chalaza was only comprised by integument derivatives. Fusion of the nucellar and integumentary bases (unitegmic crassinucellate ovules of Betulaceae, Fagaceae have a partial fusion) resulted in mesochalazal ovules. Ancestral bitegmic crassinucellate ones probably gave rise to a variety of monocot and dicot ovules, undergoing independent developmental pathways. Major directions of subsequent evolution were associated with the emergence of medianucellate ovules. Evolution in monocots presumably proceeded through medianucellate ovules with multilayered lateral and basal nucellar regions. Ovules, which are crassinucellate at early developmental stages, gradually become secondarily medianucellate due to collapse of the parietal tissue (Agavaceae, Butomaceae). Evolution in dicots proceeded from bitegmic crassinucellate ovules to unitegmic tenuinucellate ones characterized by complete nucellus degeneration prior to fertilization and endothelium formation (Alangiaceae, Cornaceae).

P0491. Phenology of *Plathymenia reticulata* (Leguminosae - Mimosoideae): characterization and variability in patterns within and among populations from areas of Brazilian Cerrado, Atlantic Forest and transitional sites between biomes

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Plathymenia reticulata is a Brazilian tree that occurs in environmentally different biomes: Cerrado (savanna vegetation) and Atlantic Forest. Phenology variability within and among populations from these biomes and in transitional zone was accessed during 15 months in ten populations from Minas Gerais State. Trees were monthly evaluated according to vegetative (leaf fall, leaf flush and mature leaves) and reproductive phases (floral buds, flowers, immature fruits and seed dispersal). In general populations showed similar phenological behavior but there were differences, related mainly to leaf fall, a process that starts months earlier in dryer areas and latter in humid sites. Most of phenological diversity was due to differences among individuals within populations and only about 10% of the total diversity was attributed to differences among biomes. In addition, the flowering periods were similar in the biomes, suggesting that populations may inbreed, and so do not contrary the new taxonomic proposal that suggested the existence of only one species of *Plathymenia* and not a species from Atlantic Forest and another from Cerrado as previously considered.

P0492. Structural organization of dicot and monocot embryos and identity of some its types (in relation to monocotly problem)

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New classification of angiosperm embryo types is suggested on the base of developmental patterns of their axial and appendicular organs and morphogenetic activity at germination. The ancient embryo type (basal dicots) arose as a result of polymerization of first leaf organs (cotyledons) in one node of axis. Two main, derived from the ancient, embryo types - "typically dicotyledonous" and "typically monocotyledonous" ones, characteristic for most of dicots (eudicots) and monocots, respectively, are principally distinguished not only by cotyledon number but also by complex of known characters. Specific manifestation of types, deviating from the main ones, within both classes correlates with the change of cotyledonary apparatus structure. The analysis of identity of different types allows understand some aspects of monocotly origin: appearance of monocotyledony in some basal dicots (via syncotly) is true but not pseudomonocotyledony; dicotyledonous type in some monocots (reliable showed for *Agapanthus*, *Cyrtanthus*, *Allium*, *Dioscorea*) displays the traces of mentioned above polymerization but does not cause principal changes in embryo structure.

P0493. Tree inventory from the north-east dry forests of the Venezuelan Guayana

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Deciduous forests represent the most endangered and unknown tree ecosystems in the Venezuelan Guayana. Inventory of the trees since was carried-out in forests communities around 10 km from Upata city (Bolívar state, 8°00' Lat. N, 62°23' Long. W, 360-680 m asl; 1.166 mm/year). A list with 295 trees species (including 5 Arecaceae and 2 Poaceae) and comments on the floristics are given. 24 % of species are legumes, 6 tree species are novelties for the Venezuelan Guayana, and 24 % of all tree species are found in the Caribbean region. Orographic stability of the Guiana shield hillside, the relatively dry nonextreme and narrow ecological gradient, could explain the presence of few endemic species at the regional level (3,5 %) and at local level (1 %) found up to now.

P0494. The ecology of the plant of the genus *Cirsium* on the border of its distribution in the Russian plain

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The ecology of 13 species of *Cirsium* and 4 hybrids has been studied in the central, northern and eastern part Russian plain and Pre-Ural in the years of 1983-2004. Several thistle species of the Russian plain are growing on the borders of their distribution; some hybrids and endemic species are distributed in this region only.

The *Cirsium* plants in the local populations on the border area have: 1) smaller height, smaller number of flower heads per specimen, than plant growing in the centre of their distribution; 2) many species of *Cirsium* form hybrids among themselves in these populations; 3) the plants are infested by more unspecialised insects.

4) The fauna of specialised phytophages of *Cirsium* is more diverse in the centre of the species distribution.

Taking into account the absence of specialised phytophages in many cases of introduced plants and on the grounds of the studied fauna of phytophages of *Cirsium* and its hybrids we conclude that *C. palustre*, *C. incanum* and *C. esculentum* are not native in the investigated regions. This contradicts the view of many botanists about the formation of the modern thistle flora of this region and on the Russian plain

P0495. Neither inbreeding depression nor sex allocation appears to maintain the sexual polymorphism in *Opuntia*.

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Several models have been proposed for explaining the evolution of unisexuality from hermaphroditism. Comprehensive studies testing the predictions of the models are needed. *Opuntia robusta* is a sexually polymorphic cactus with hermaphroditic, dioecious, and trioecious populations. We compared the vegetative and reproductive performance of males, females and hermaphrodites in a trioecious population. In consistency with the sex allocation

theory, the potential reproductive output of the functional sex was higher in unisexuals when compared to that of hermaphrodites. Moreover, a disproportional increase found males is required for unisexuals to invade an original hermaphroditic population with the features of hermaphrodites: prior- and overriding selfing, no detectable pollen discounting nor inbreeding depression. These results might explain the existence of dioecious populations, but in our population, female seed output had a similar mean but a higher variance than hermaphrodite's. Vegetative changes related with the sexual polymorphism, and unreliable pollination services appear to be the major determinants of the observed polymorphism.

P0496. Population strategy of the genus *Allium* species

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Onions hold different positions in phytocoenosis. They may dominate or be subordinate. One or other role in the community results from population biology.

Ontogenesis, propagation by seeds, vegetative reproduction, ontogenetic structure and coenopopulation density were studied in 40 species of the genus *Allium* L. in different habitation conditions in Siberia, Kazakhstan and Middle Asia. Six types of ontogenesis were described. The correlation between the way of propagation and the type of ontogenesis was established. The ontogenetic spectra and types of self-preservation of the coenopopulations were characterized.

Using concepts of plant strategies (Ramensky, 1938; Grime, 1979, 2000; Rabotnov, 1985; and Smirnova, 1987), we identified several population strategies in onions on the basis of studied specific features of species biology. They were named by the dominance of one of the properties: competitive ability, reactivity and tolerance. Competitive, reactive, tolerant and tolerant-reactive species were determined among the species of the genus *Allium*.

P0497. Predispersal seed predation and its influence on the fitness of the rare carnation *Dianthus hinoxianus* Gallego

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Phenology, breeding system and seed predation of *Dianthus hinoxianus* Gallego (Caryophyllaceae), an endemic hemicytrophite specie in shrublands on paleodunes of SW Spain, were studied. *Dianthus hinoxianus* is a gynodioecious and self-compatible species but natural self-pollination is rare due to marked protandry. Nectar production was not significantly different between sexual phases. Flowering takes place in June-August and flowers are pollinated by diurnal and nocturnal lepidopteran, mostly hawk moths (*Macroglossum stellatarum* and *Hyles lineata*). Lepidopteran larva, born from eggs laid inside the flowers, fed on seeds inside the capsules. An experiment of selective exclusion of pollinators showed that eggs were laid mainly by night. In this study, of a total of 42 monitored patches about 97.6% had at least one flowerdamaged and 54.9% of the flowers (N=855) were damaged. All the populations showed a high rate of predation with significant inter- and intra-population differences.

P0498. What is the special floral scent of willows?

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The genus *Salix* (willows) is composed of dioecious woody plants with a high taxonomic and ecological diversity. Inflorescences of most willow species show adaptations to insect pollination. The pollinators are believed to play a role in limiting interspecific gene flow. The floral and pollen scents are assumed to be the key components for attracting the pollinators. However, only few data are available characterising volatile compounds of willows and their functions.

Floral odours of different willow species were analysed using dynamic-headspace MicroSPE methods.

Investigations on three willow species (*Salix caprea*, *S. cinerea* and *S. alba*) revealed benzoids (e.g. 1,4-Dimethoxybenzene) and isoprenoids (e.g. -Pinene, D-Limonene) as main compound classes of floral scents. The benzoid compound 1,4-Dimethoxybenzene could only be found in *S. caprea* and *S.*

cinerea, but not in *S. alba*. The role of different volatiles in attracting specific pollinators is discussed.

P0499. Taxonomy and reproductive biology of *Cerasus fruticosa* (Rosaceae) in Slovakia

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Cerasus fruticosa (Pall.) Woronov is a rare species on the territory of Carpathian basin and the result of hybridization with cultivated taxa of genus *Cerasus* the *C. x eminens* (Beck) Buia and *C. x mohacsyana* (Kárpáty) Janch. were confirmed in our study. The process of the microsporogenesis and microgametogenesis of *C. fruticosa* is normal, the level of degenerated pollen grains of both hybrids was rather high (20 - 60%). Germination of pollen grains *C. x eminens* was 0 % and *C. x mohacsyana* 20%. Two ovules differentiation in each carpel of these taxa and degeneration of the secondary ovule seems to be genetically programmed. Sometimes we observed degeneration of both ovules. We didn't find viable ovules in May in the pistils of the *C. x eminens* and production of any seeds during years 1998-2004 were noticed. In the flowers of *C. x mohacsyana* from 2 to 5 carpels was formed and some irregularities in development of female gametophyte has been achieved.

P0500. Size dependent gender diphasy and pollen movement in *Arisaema serratum* (Thunb.) Schott (Araceae)

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Arisaema serratum possesses a pitfall-trap flower pollination system. However, little is known about the efficiency and pattern of pollen movement in this species. Thus, the aims of this study are: (1) to determine the paternal parents of the seeds and (2) to elucidate pollen movement in a natural population.

Seeds were collected from the natural population of *A. serratum* in 2001 at Horigane, Japan. Small midges were trapped in female spathe tubes during the flowering period. Paternity analysis using microsatellite markers was performed. We found that: (1) seeds in a fruit were fertilized by multiple sires; (2) a few males contributed to a great extent as sires; (3) distance from a female was not a factor in the inequality of reproductive success among males; (4) male reproductive success was not correlated with its size.

We conclude that pollen carryover and the trap-flower pollination system are most likely to result in multiple paternity and inequality in male success. This study sheds light on the pollination syndrome in trap-flower pollination, or provides a basis on which to test the size-advantage hypothesis in *A. serratum*.

P0501. Genus *Dipcadi* Medik. (Liliaceae) in Indian subcontinent: cytogeography and speciation.

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Genus *Dipcadi* Medik (Liliaceae) is represented by about 9 species in Indian subcontinent of which 6 occur in the northern Western Ghats. The remaining three Indian species namely *Dipcadi erythraeum* Webb. et Berth., *D. reidii* Deb and Dasgupta and *D. serotinum* (L.) Medik. are distributed in hilly regions of arid zones of Rajasthan, in West Himalaya and in Western Himalaya and Nepal, respectively. The present study reports on the distribution, field status and karyomorphology of 8 Indian species of *Dipcadi*. Among Indian species of *Dipcadi*, *D. montanum* with 2 median, 5 submedian and 3 subterminal chromosomes has comparatively primitive karyotype. Three *Dipcadi* species with 2n=12 have 3 submedian and 3 subterminal chromosomes. Among Indian species of *Dipcadi*, highest somatic chromosome number, i.e. 2n=22, has been observed in *D. erythraeum*. The paper discusses cytogeography, interrelationship and speciation of *Dipcadi* species in India and the northern Western Ghats as one of the likely area for diversification of the genus in Indian subcontinent.

P0502. Irregular patterns of flowering and fruiting and androdioecy in *Phillyrea latifolia* L. in Israel

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Frequency, rate and intensity of flowering and fruiting in the two sex types of the androdioecious shrub *Phillyrea latifolia* L. were monitored. High inter-annual fluctuations in rates and intensity of flowering and fruiting occur. Functional male individuals (normal ovary in the flower, smaller stigmata, no fruit production) flower more frequently and intensively than hermaphrodites. Many individuals avoid flowering at all. Disturbance (shrub removal together with grazing) increases the rate, frequency and intensity of flowering. Fruiting rates of hermaphrodites are generally low and the fruit yields vary highly among individuals. Many individuals do not produce fruits at all, while only few produce significant fruit yield. Observations were made on *Probrugmanniella phillyreae* (Diptera, Cecidomyiidae) which attacks the ovaries both in functional males and hermaphrodites and causes the formation of distorted ovary galls instead of normal fruits.

The role of climatic gradient, disturbances and insect damage as selection factors behind the androdioecy and the irregular patterns of the reproductive traits in *P. latifolia*, is discussed

P0503. Pollen flow within and between subpopulations of *Magnolia stellata*

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Magnolia stellata (formerly also known as *M. tomentosa*) is a deciduous, clone-forming tree of the Magnoliaceae. It is now a threatened species, classed as "vulnerable" in the "Red Data Book of Japanese vascular plants". Disappearance and / or fragmentation of populations disturb gene flow and accelerate differentiation among populations. In order to provide baseline information for the genetic diversity in *M. stellata*, pollen flow within and between subpopulations was investigated by using 10 microsatellite markers developed in *M. stellata* and *M. obovata*. Paternity analysis was conducted for 418 seeds collected from nine maternal genets in a subpopulation located at the center of a population. The population was composed of eight subpopulations where a total of 313 flowering genets were growing. Out of the 418 seeds, 97.4 % were offspring sired by paternal genets within the subpopulation and the remaining 2.6 % were by paternal genets in the other subpopulations, indicating predominance of short-distance pollen flow in the population. The paternal genets were mostly large-sized, with long flowering periods, and located near the maternal genets.

P0504. Floral capitate hairs of *Veronica* (Scrophulariaceae): structure and function

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Glandular hairs densely cover gynoecium in some species of *Veronica*. The hair consists of a long multicellular stalk and a large two-celled head. The TEM study showed that lipids are accumulated in the head cells. At the beginning of blossoming, before nectar secretion, they penetrate in the cell wall, which changes its structure in these places. It becomes looser, the areas between fibrils of cellulose contain similar to lipids substances. Lipids come through and accumulate under cuticle, making small swelling. After cuticle rupture oil drops evaporate. The exudation of lipid drops can happen either several places of the same cell (eg. *V. longifolia*, *V. teucrium*) or in one place - on the top of the hair head (*V. officinalis*). Thus the glandular hair apparently play the role of osmophores. It is interesting that we are the first to discover such type of glandular hair on the large nectar disk in *V. officinalis*. In this case nectaries play the double exudation role - evaporation of smelling essence first by hair and nectar secretion through stomata in future.

P0505. Parasitism effect by bruchids (Coleoptera-Bruchidae) on seed germination of *Mimosa* species (Leguminosae-Mimosoideae) in Mexican semi-arid ecosystems

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Genus *Mimosa* is well spread over Mexico with 110 species. Some *Mimosa* species occur in semi-arid ecosystems being dominant

within their communities and forming resource islands, which favor soil and biodiversity conservation. These species are used by local people. Eight species and two varieties were sampled, all endemic to Mexico. These species produce ca. 4000-15000 seeds/plant; although, a high percentage of seeds is not viable (30%-75%) due to bruchid parasitism. Germination capacity was compared between healthy and infected seeds. A high seed germination was recorded (>90%) either in the laboratory and in the field, optimal temperature was 25°C. However, infected seeds only reached <20% of germination. There is an inverse relation between seed coat thickness and seed vulnerability to bruchid infection. A bruchid data base is being developed, including 12 species of genus *Acanthoscelides* and 3 of genus *Stator*, that infect ca. 50 Mexican *Mimosa* species. This study contributes to the understanding of *Mimosa* reproductive biology and is an attempt to develop a method to evaluate the impact of these coleoptera seed beetles against native *Mimosa* populations.

P0506. Autecology of *Isoëtes echinospora* and *I. lacustris*, critically endangered species of glacial lakes in the Bohemian Forest, Czech Republic

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Isoëtes echinospora and *I. lacustris*, submerged lycopodiophytes, are glacial relicts and critically endangered species of the Czech flora. In the Czech Republic, they occur only in two strongly acidified and anthropically disturbed lakes of the Bohemian Forest, Cerne and Plesne. Lack of information on viability of the populations and speculations about their limited reproduction raised a need for recent underwater investigations of reproduction biology and autecology. Quillworts are periodically monitored on permanent study plots and their 3D delimitation within populations and biometric characteristics are assessed. Experiments on survival ability in other parts of the lakes and simulations of repatriation are conducted in plant nurseries. Environmental conditions are recorded by dataloggers and sediment and water are analysed. In laboratory experiments, the influence of environmental factors on spore germination and sporophyte development are studied and temperature, pH and Al limits were already established. In the roots of both quillwort species sampled in the lakes, colonisation by arbuscular mycorrhizal fungi was frequently observed.

P0507. *Centaurea phrygia* agg. in central Europe: morphological, karyological and isozyme variations and introgressive hybridization

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Three taxa were recognised within *C. phrygia* agg. on a basis of multivariate morphometric analysis and chromosome counts: *C. pseudophrygia* (diploid), *C. stenolepis* (diploid) and *C. phrygia* s.str. (diploid and tetraploid, the ploidy levels seem to be geographically separated and no mixed populations were found). Morphologically intermediate populations, which have not been reported by recent authors, were found: (1) between *C. pseudophrygia* and *C. stenolepis* and (2) between *C. phrygia* (tetraploid cytotype) and *C. oxylepis* (from *C. jacea* agg.; tetraploid). They probably originate from an introgressive hybridization. Results of an inquiry of their distribution and karyological and isozyme variations support this hypothesis. The intermediate populations occur in contact zones of the putative parental species. They have the same number of chromosomes as the putative parents, which is an unavoidable condition for repeated hybridization in *Centaurea*. Isozymes were studied for *C. pseudophrygia* - *C. stenolepis* pair: the pattern of variation of isozymes within possibly hybridogenous populations is intermediate between the putative parents.

P0508. Extensive self-pollination in an endangered population of *Pinus parviflora* var. *parviflora*

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Plants of *Pinus parviflora* var. *parviflora* in the Boso Peninsula, Japan have been rapidly decreased in number in recent decades. The population is severely fragmented, and only 80 plants survived at present. For the purpose of conservation, we examined pollen dispersal pattern within and among the fragmented patches by using seven microsatellite markers. DNAs of all mature plants and megagametophytes and embryos of 72 seeds from twelve maternal plants were extracted and their genotypes were determined. Paternity exclusion analysis revealed that extensive self-pollination occurred in this population; among 72 seeds, 67 (93%) were selfed ones, and the other five were outcrossed ones (one intra-patch, two inter-patch, and two by unknown paternal plants). The low outcrossing rate (7%) seems to be extraordinary, because most of pine species have been considered to be predominantly outcrossing. Limitation of pollen available for outcrossing was also suggested by a low full-filled seed rate (17%) in this population. Possibly, this population is involved in extinction vortex by the decrease in number and inbreeding.

P0509. Reduction of geitonogamy: Flower abscission for departure of pollinators

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Overproduction of flowers increases the attractiveness of a plant to pollinators, but results in increased geitonogamy. Problems of geitonogamy are probably most severe in large trees because they produce large numbers of flowers and because of their overall size. In general, flower abscission has been considered to be an event subsequent to the overproduction of flowers. *Tilia japonica* is a self-incompatible, insect-pollinated tree. *T. japonica* produces morphologically perfect and protandrous flowers, however some of the flowers are aborted before the female phase to regulate flexibly its sex allocation. We observed pollinator behavior in *T. japonica* and found that pollinators sequentially visited inflorescences within a tree; however, when they met with flower abortion by abscission, they were apt to move long distances and leave the tree. We propose that plants may utilize flower abscission as a method for regulating the movements of pollinators by disturbing them in order to prevent geitonogamy.

P0510. Arrangement of multiple sequence variants at telomeres of Hyacinthaceae

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Telomeric sequences are considered as highly conserved across a broad phylogenetic spectrum. In many higher plants Arabidopsis motive (TTTAGGG)_n is common. However, there is an evidence that typical plant telomeric motive is absent in several species. A group of monocotyledonous plants Asparagales, forming a distinct clade in phylogenetic analyses, was previously reported to lack typical "Arabidopsis" type telomere. According to the type of their telomeric sequences Asparagales order can be subdivided into three groups - plant type (e.g. orchids), human type (e.g. asparagus) and unknown type (e.g. onion).

Telomerase of Hyacinthaceae plants species - e.g. *Ornithogalum muscari*, *Scilla* - synthesize human-type telomeres with high error rate in vitro. Using FISH, fiber-FISH, and Southern hybridisation, we characterize structure of telomeres in these plants by analyzing co-localisation between major (human-type) and minor (Arabidopsis-type or Tetrahymena-type) sequences.

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P0511. Spatio-temporal fluctuations in sex ratio which cause pollen limitation in the dioecious herb *Antennaria dioica*

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Dioecious plants may be more sensitive to habitat fragmentation than hermaphrodite plants due to the spatial separation of the sexes. This is in part dependent on the pollination system of the plant. We have studied *Antennaria dioica*, which has a potentially good pollen transfer system with many types of insects as vectors (eg. Anthomyiidae, Calliphoridae, Geometridae). We monitored the phenology pattern of the species for three years at a local scale, and during one year at a regional scale. We also conducted a pollination study to assess the impact of isolation between male and female plants on seed set. Our results suggest that there are large fluctuations in sex ratio and flower number between years. Small populations of the species tend to have a more skewed sex ratio, compared to larger populations. We also found that some patches of the species, but not all, where pollen limited and thus had a lower natural seed set. This variation in pollen limitation could be explained by the sex ratio and abundance of males within a short distance from the patch. Our results have implications for the conservation and management of dioecious and other obligate outcrossing plants.

P0512. 08 The pollination biology of *Ilex* species in Hong Kong, China

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The pollination biology of fourteen native *Ilex* species in Hong Kong was studied. All species flowered annually between March and June. Male plants produced 2.4-11.2 times as many flowers on an 8-mm diameter branch as female plants. Nectar amount and chemistry varied among species and between sexes. *Apis cerana* accounted for >87% of diurnal flower visits for all species and male flowers received more visits than female flowers. Minor diurnal visitors included other bees, wasps, butterflies, ants and beetles. Moths and beetles were the major nocturnal visitors, but the visitation rate was very low. Across species, the *Apis* visitation rate per flower was strongly related to the estimated total number of flowers on the plant in both male and female plants, but was not related to nectar production or flower size. The percentage fruit set varied widely between species (8-73%) but was not significantly related to *Apis* or total visitation rates.

P0513. "The Uncertainty Principle" of Molecular Systematics

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Molecular clock principle in the Neutral Theory of Molecular Evolution is applied to analyze evolutionary relationship of extant taxa by comparing their DNA sequences. This methodology has been a fashion in molecular systematics, but it has inherent limitations similar to predicaments in Classic Mechanics, which is subsequently called an Uncertainty Principle in Quantum Mechanics. The Uncertainty Principle is also suitable for DNA sequence comparison because the number and location of nucleotide variation can be sequenced, but their occurring time and influence on structural function cannot be measured. Differential accumulation of nucleotide variation from extant taxa only reflexes relative temporal distances from their own ancestors. If there were theoretically proportional relationships with differential accumulation of nucleotide variation among different taxa, it would create comparatively feasible results to elucidate their evolutionary relationships. However, extant taxa are not actually diversified from their ancestors by proportional time, so DNA sequencing results cannot mirror true evolutionary relationships among taxa.

P0514. Morphology and anatomy of the fruit and seed in development of *Piper crassinervium* H.B. & K. (Piperaceae)

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The purpose was describe the morpho-anatomy of the fruit and seed of *P. crassinervium*. The material consisted of spikes in different stages of development. Analyses were made in fresh and fixed material. Permanent and semi-permanent slides were prepared according to standard procedures. The erects spikes are displaced in a opposite position of the leaf. The achlamydeous and bisexual flowers grow in the axile of bracts. The uniseriate epidermis of the bracts presents stomata, trichomes and with the oils cells. The mesophyll is homogenous and with the oils cells.

The unilocular and superior ovary presents sessil stigmas. The unic ovule, with basal placentation, is orthotropous, bitegmic and crassinucellate. The outer integument shows three or four cell layers, and the inner four. The micropyle, is filled by an obturator. The mature seed is orthotropous, endotegmic and perisperm. The ovary wall is constituted by uniseriate epidermis. The parenchymatic ovarian mesophyll presents elongated radially cells and the oils cells. The ventral epidermis is uniseriate. The pericarp in mature fruit is similar to the ovary wall during all development. (CAPES/FUNDUNESP)

P0515. Anatomical and ontogenetical studies of the corona, operculum and limen in species of *Passiflora* L. subg. *Decaloba* (DC.) Rchb. (Passifloraceae).

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It carries out an anatomical and ontogenetical study of the corona, operculum and limen of *Passiflora capsularis* L. and *P. suberosa* L. (subg. *Decaloba*), besides discussing the origin of those structures. This subgenus is characterized by the flowers frequently small, solitary or in pairs, 5-merous, hypanthium campanulate or dish-shaped, with corona uni or bisseriate, disposing the filaments in successive circular series. In *P. capsularis* the flowers have sepals and petals and the only filaments series of the corona and the operculum have origin in the hypanthium, with the filaments united in the base as a annular membrane. In *P. suberosa* the flowers have only sepals and both filaments series of the corona and the operculum originate simultaneously from the hypanthium. In both species only the corona has vascular bundles. The operculum is the most intern verticilo of the corona and it resembles a annular membrane, folded into pleats, with the apex fimbriate or denticulate. The limen, a annular membrane, is the most intern part of the corona and it originates close from the base of the androgynophore.

P0516. Complexity of the plant surface function in the multitrophic system: plant surfaces as environment for the predatory mirid bug *Dicyphus errans* Wolff (Heteroptera, Miridae)

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The common function of plant trichomes is the protection of plants against water loss, solar radiation and phytophagous insects. Many insects may be deterred or killed by hooked and glandular trichomes. Some insect species, f. e. the omnivorous *D. errans*, are associated with pilose plant surfaces. This bug shows morphological (slim body, long slight legs, elongated curved claws) and behavioural (way of locomotion) adaptations which allow close interactions between hairy plant substrates and the bugs. To study the influence of different types of plant leaf surface on the attachment of the bugs, screening experiments and force tests were conducted. The screening of leaf surfaces of 40 plant species belonging to 25 families showed successful attachment of bugs on plant substrates covered with glandular and non-glandular trichomes. In traction force tests on 15 leaf surfaces, insects also performed better on hairy substrates. The force correlated significantly with trichome length and diameter. The results show that hairy plant surfaces may not only protect the plant against herbivorous insects, but also provide the suitable environment for predatory insects.

P0517. Isolation mechanisms between *Heloniopsis orientalis* and *Heloniopsis breviscapa* (Liliales: Melanthiaceae)

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Heloniopsis orientalis and *H. breviscapa* grow sympatrically in Kinki and Shikoku Districts, Japan. In this study, we investigated two sympatric populations of them using morphological, plastid DNA and microsatellite markers in order to clarify how the two species are isolated. At the population in Kinki, *H. orientalis* and *H. breviscapa* var. *flavida* proved to be isolated primarily by ecological and floral factors. However, a vegetative character and molecular

markers demonstrate a certain level of introgression of a trait from *H. breviscapa* var. *flavida* into *H. orientalis*. Floral characters (flower color and nectary position) show no introgression, and may be considered to be under selective control, although their floral morphologies are primarily basic among monocot ones without any marked appendages such as a spur. At the population in Shikoku, seasonal factors may be decisive in isolation between *H. orientalis* and *H. breviscapa* var. *breviscapa*. Their flowering periods were not overlapped with one-week gap in 2004. Thus, isolation mechanism between *H. orientalis* and *H. breviscapa* varies from locality to locality and / or from variety to variety.

P0518. On the phylogenetic continuity of the telome of Rhyniophyta and the phytomer of monocotyledons

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The segmented body of plants is organized on a principle: the following phytomer arises from the previous one. The initial unit - a telome in Rhyniophyta and an embryo (phytomer) in Monocots - repeat itself many times, specializing along the shoot into growth-units of 3-5 types. The telome is an axis with 2 initials at the upper end and 2 at the lower end. The prothallus of Psilotum and Tmesipteris are autonomous 5-axial mesomoids. The phytomer of Monocots (an evolutionarily modified 5-axial mesomoid with anisotomous right and left halves) has besides the axis four appendicular developments: on the innovative half, a leaf (a homologue of the originally terminal sporangium), and a root (a homologue of the rhizomoid); new phytomers accrue on the retarded half: from the upper initial - "monopodially", from the lower (a lateral bud) - sympodially. The lateral bud, like the rhizomoid, is orientated downwards. Therefore the prophyll of a sympodium is always adorsalized and has 2 keels. The dichopodial nature of the phytomer determines the invariably lateral position of a leaf primordium. The unifaciality of the sporangium is forerunner of the cotyledonary sheath of Monocots.

P0519. Myrmecochorous diaspors as baby-food for ants

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Ants carry myrmecochorous diaspores usually into their nests where the lipid-rich appendage of the diaspores (elaiosome) is consumed. Here we present a study on the distribution of *Corydalis cava* (Fumariaceae) elaiosomes between the larvae and the working caste of laboratory colonies of *Myrmica rubra* (Myrmicinae: Formicidae). In a combined ¹³C and ¹⁵N labelling experiment elaiosomes proved to be a far more attractive diet for the larvae than Bhatkar diet, contributing 86.8% ± 2.0 of the daily nitrogen and 79.2% ± 2.8 of daily carbon incorporation of larvae. Workers incorporated less carbon and nitrogen based on dry body mass during the experiment - for nitrogen these proportions were lower by a factor of 1.6 to 13.1 and for carbon by a factor of 1.8 to 10.3. Laboratory ants stored diaspores within their nests for up to four days. We therefore hypothesize that elaiosomes may not only be a major food source for growing larvae, but also an important nutrient buffer for ant colonies under natural conditions where food supply might be highly discontinuous.

P0520. Sexual reproduction of *Espeletia grandiflora* var. *multiflora* in a high andean paramo of Colombia

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Some aspects of reproductive strategy of *Espeletia multiflora* var. *grandiflora* were studied. Three patches of *Espeletia*'s plants characterized by agricultural activities in the surroundings, and located in a high andean páramo of Colombia were chosen. The field experimental phase was made since July to December in 2003. Four hypotheses were tested: 1. Disturbances can reduce the seed production in native ecosystem; 2. Disturbances can induce increases in viable seed production by autogamy; 3. There is a direct relationship between plants' height and investments in reproductive structures; 4. The time expected for phenological phases is different in accord with the conservation state of the place. Results were compared with another study made in the Chingaza's Paramo (Fagua 2002), characterized by better degree of conservation. For this place was reported lower viability

percentage, higher autoincompatibility index, lower seed plant production, and shorter phenological phases that in Chingaza's Paramo. Differences of conservation degree, climate, and genetic composition of *E. grandiflora* between both places can explain the different results.

P0521. Is *Cycas revoluta* (Cycadaceae) anemophilous or entomophilous ?

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Cycas revoluta has been considered anemophilous. However, we have encountered a few indications of entomophily, such as the presence of a strong odor emitted by male and female cones at the time of pollination. In this paper we report the results of our experiments to determine the method of pollination. Pollen exclusion experiments were devised to test if wind is a sufficient vector effect pollination on Yonaguni Isl. (Okinawa, Japan), where thousands of individuals grow and produce fertile seeds under natural conditions. Insect exclusion resulted in a notable decrease in seed production in most cases, but a few female plants growing close to male cones bore as many seeds as the controls. Airborne pollen grains were few except within a 2 m radius of male cones. Anemophily, therefore, appears effective only over short distances. In contrast, various kinds of insects were collected from female cones. Among them, one species of Nitidulidae (Coleoptera) bore pollen grains of *C. revoluta* on their body. Consequently, insects appear to play an important role in the pollination of *Cycas revoluta*, especially in transferring pollen grains over long distances.

P0522. Adaptation of plants to nutritional needs of diaspore-dispersing ants

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The nutritional composition of the appendages of the diaspores (elaiosomes) compared with the respective seeds has received little attention so far. We therefore analyzed seeds and elaiosomes of 15 Central European ant-dispersed (myrmecochorous) plants for their content of lipids, amino acids, soluble carbohydrates, proteins and starch.

In most of the seeds and elaiosomes, lipids were the main constituent. While seeds had a significantly higher protein content (6% in seeds vs. 3% in elaiosomes) and on an average more lipids (26% in seeds vs. 20% in elaiosomes), elaiosomes contained significantly more free amino acids (7% in elaiosomes vs. 1% in seeds).

Although the overall nutrient quality was similar in diaspores and elaiosomes, the specific composition of individual fatty acids, amino acids and carbohydrates differed markedly between elaiosomes and diaspores. The fatty acid pattern in seeds were taxon-specific while it was homogenous in elaiosomes.

This suggests a directed adaptation of the elaiosomes' composition to the ants' nutritional needs.

P0523. Floral Biology of three páramo rosettes in the Eastern Andes of Colombia

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We described the floral biology of *Espeletia barclayana*, *Espeletia argentea* and *Espeletopsis corymbosa* at the Páramo de Guerrero (3200-3600m; 5°04' N, 74°06' W) in the Eastern Andes of Colombia. The three species are monoecious, their capitula are protogynous and last about 15 days. The female flowers are ligulate and peripheral, and are receptive since the second day of anthesis. They live as long as the capitulum and do not produce nectar. The male florets constitute the central disc and initiate anthesis one or two days after female ones. They open centripetally, last one day and produce pollen and nectar. *E. barclayana* produces 140 male florets and 82 female flowers in average in a head, *E. corymbosa* produces 37 male flowers and 25 female and *E. argentea* produces 67 male florets and 46 female flowers. *E. corymbosa* presented the highest number of inflorescences per plant and heads per inflorescence but shows the smallest heads. On the other hand, *E. barclayana* produces the lowest number of inflorescences per plant and capitula per inflorescences but has the largest heads. These results may

indicate a trade off between size and number of capitula for these species.

P0524. Reproductive systems of three páramo rosettes in the Eastern Andes of Colombia

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We studied the reproductive system of *Espeletia barclayana*, *Espeletia argentea* and *Espeletopsis corymbosa* at the Páramo de Guerrero (3200-3600m; 5°04' N, 74°06' W) in the Eastern Andes of Colombia. *E. argentea* and *E. corymbosa* were partially self-incompatible (ISI= 0.356 y 0.304, respectively) but *E. barclayana* was strongly self-incompatible (ISI=0.0114). The proportion of viable seeds was higher in cross pollinated heads than in self pollinated, as it was expected. In the control treatment (pollination under natural conditions) *E. barclayana* presented the lowest proportion of viable seeds and *E. argentea* the highest. On the other hand, in the cross pollination treatment (artificial) *E. barclayana* produced the highest proportion of viable seeds and the other two species (*E. argentea* and *E. corymbosa*) produced a lower proportion. This shows us that in natural conditions *E. barclayana* has limitations for its reproductive success. The pollination efficiency, measured as the proportion of female flowers in a head that became achenes, was relatively low for the three species, ranging from 38 to 65%, indicating pollinator limitation or their low efficiency.

P0525. Critical re-evaluation of the function of 1,2 diolein as trigger for ant-dispersal

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The trigger of myrmecochorous diaspore dispersal is still debated. The currently most accepted hypothesis is that 1,2 diolein, a diacylglycerol of oleic acid causes stereotypical carrying behaviour. We investigated the mechanical and biochemical background of three myrmecochorous plants from Central Europe (*Corydalis cava*, *Galanthus nivalis*, *Knautia dipsacifolia*) and four colonies of two ant species (*Formica fusca*, *Myrmica rubra*) in experiments using natural diaspores and artificial seeds/elaiosomes with the respective extracts. Elaiosome extracts were compared to seed extracts of the same species. Seed extracts were collected, but without clear preference. A general preference for mixtures of elaiosome extracts was observed, followed by the fat extract, amino acids/sugar extract and, finally, protein extract (descending sequence).

The significant preference of elaiosome extract mixtures over the fat fraction and pure 1,2 diolein, indicates that the nutrient quality and complex chemical composition of the elaiosomes and not a single chemical compound is essential for carrying behaviour of the ants and thus an important factor in the mutualism.

P0526. The comparative carpology and taxonomy of the family Celastraceae R.Br. s.l.

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The complex comparative morphological and anatomical study of structure of fruits and seeds of 108 species from 39 genera of the family Celastraceae is conducted. It was determined taxonomical importance of features which in most cases to well correlated with types of living forms, texture of stomatal complex, structure of flowers and pollen granules.

Then the data was processed by cladistic and SYNAP-methods. The following conclusions was draw. 1) The family Celastraceae R.Br. it is necessarily consideration in wide sense, including Hippocrateaceae A. Juss. 2) The tribe Lophopetaleae Loes. is formed one clade with Hippocrateaceae. 3) Subfamily Tripterygioideae Loes. is monophyletic and monotetic taxon with one important feature - one-seeding nut-like fruit. 4) *Sarawakodendron* with *Brassiantha* and *Dicarpellum* are related and an isolated genera in the family Celastraceae, represents an independent group within the family - subfamily *Sarawakodendroideae* I. Savinov et Melikian. Moreover, molecular data best of all to be used to determine of volume and relationships of separate genera.

P0527. Style diversity in the family Cactaceae

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Our goal was to describe and compare the diversity of stylar anatomy in the Cactaceae family to gain insight in its evolutionary significance. Various developmental stages of floral buds and flowers in anthesis of more than 50 species belonging to the Cactaceae subfamilies were studied through sections and scanning electron microscope. Among the distinctive characters are the papillose epidermal cells as for *Mammillaria* and *Gymnocalycium* species and the different organic and inorganic compounds occluding the epidermal cells. Although the transmitting tissue has similar type of cells, its width and shape varies among genera. In addition, the centre of the style is open conforming a stylar canal along its length in members of Opuntioideae and Cactoideae. A semi-solid center was observed in species of *Myrtillocactus* and a solid one in *Mammillaria* species. When the stylar cavity is present, papillae with dark-staining deposits or lacking is distinctive. Moreover the stigma has one vascular bundle per lobule, with collenchyma cells in few species. A number of style and stigma anatomical characters with phylogenetic signal will be highlighted.

P0528. Diversity of flower structure as criterion of systematics and phylogeny of Aizoaceae.

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Undoubtedly, flower evolution in Aizoaceae occurred in direction of epigyny formation which at most specialized Ruschioideae was accompanied by change of placentation type that is connected to gynoecium deformation owing to receptacles invagination. Within the family there are flowers with various degree of evolutionary specialization, which main features are ovary type (perigyny up to complete epigyny), placentation type (central-angular up to basal and parietal), androecium structure (single up to numerous) and perianth structure (simple up to complex). The degree of placentae displacement directly depends on epigyny level. So, at weak-epigynous genera the transitive type between central-angular to basal, at semi-epigynous - basal placentation type are observed. At some epigynous genera is present the intermediate basal-parietal placentation type. At most specialised epigynous genera are developed a parietal placentation with placentae arrangement extremely on peripheral ovary walls. Such variety of flower structure is a systematic attribute and may be a reliable criterion of phylogeny and a level of primitiveness or evolutionary advances.

P0529. Floral development in Calceolariaceae: Intergeneric variation and comparison with allied groups

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Floral development of the recently established Calceolariaceae was studied to clarify their controversial floral morphology and the relationships with other groups of Lamiales. 12 selected species (*Calceolaria*: 8, *Jovellana*: 3, *Stemotria triandra*) were studied from flower initiation to anthesis by means of SEM. All genera share a similar pattern of organ initiation. The four-parted calyx is the first floral series initiated, with the lobes emerging in a clear dorso-ventral succession. In *Calceolaria* and *Jovellana* the two corolla lips arise as uniform ridges. In contrast, the lower lip of *Stemotria* is bilobed, resulting in a trimerous corolla. This is paralleled by the presence of three stamens instead of two. A similar architecture was also found in teratological flowers of *Calceolaria*. These results suggest that the perianth of Calceolariaceae is truly tetramerous and not derived from pentamery. These findings are in agreement with the separation of Calceolariaceae from Scrophulariaceae, suggested by recent molecular data, and with their placement in basal Lamiales, following the Oleaceae and Tetrachondraceae with clearly tetramerous flowers.

P0530. Comparative floral development in Calycanthaceae (incl. Idiospermeaceae)

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Calycanthaceae have a prominent position in basal angiosperms as they appear as sister to all other Laurales in molecular

phylogenetic analyses. Reproductive structures of representatives of the four extant genera of the family (*Calycanthus*, *Chimonanthus*, *Sinocalycanthus*, and *Idiospermum*) have never been comparatively studied. The aim of this work is to provide a comparative investigation of floral structure and development, with emphasis on floral phyllotaxis, development of androecium and gynoecium, and floral behaviour just prior to and at anthesis. Previous studies on floral phyllotaxis were all based on a single species (*Calycanthus floridus*). All studied species have a spiral floral phyllotaxis according to the Fibonacci pattern, which begins after a number of decussate organs. Correlated with spiral phyllotaxis is variability in organ number; however, stamens tend to appear in series of Fibonacci numbers (varying between taxa). Carpel differentiation in the paucicarpellate genus *Idiospermum* is different from that in pluricarpellate genera, but all genera have in common a plicate or only weakly ascidiate carpel form.

P0531. Floral and inflorescence anatomy in the Lecointea clade (Leguminosae, Papilionoideae, Swartzieae sensu lato)

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Floral and inflorescence anatomy were studied in *Exostyles*, *Harleyodendron*, *Holocalyx*, *Lecointea*, and *Zollernia* (*Lecointea* clade). Common features include: anther, ovary, petals, sepals and bracts supepidermal cells with phenolic compounds (except in *Holocalyx balansae*); tector trichomes at the bracteoles inner base and on the calyx outer surface, including the species with colleters (except in *Exostyles godoyensis*). Significant anatomical features include colleters in *Holocalyx balansae* and in *Zollernia ilicifolia* and tector trichomes in *Exostyles venusta*, *Harleyodendron unifoliolatum*, *Lecointea hatschbachii* and *Zollernia magnifica*; trichomes at the anther epidermis in *Lecointea hatschbachii* and *Holocalyx balansae*, a very uncommon characteristic; synchronic pollen development into the anthers in *Exostyles venusta*, *E. godoyensis*, *Holocalyx balansae* and *Zollernia ilicifolia* and asynchronous in *Lecointea hatschbachii*; vascular bundles surrounded by lignified fibers in *Harleyodendron unifoliolatum*. The genera of the *Lecointea* clade share some rare characteristics in Leguminosae showing an anatomical affinity among them (FAPESP).

P0532. Flower structure and its morphological transformation in Polygonaceae

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All the diversity of flower structure could be described in terms of merosity and completeness. The flower merosity is calculated as the number of tepals per whorl, and depends: on the meristem size and shape, and on the size of presumptive tepal primordia, related to meristem, at the moment of spatial pattern formation in the flower. Merosity increases as a result of floral meristem diameter enlargement and/or of size reduction of tepal primordia. The number of sites for stamen positioning is correlated with the tepal number. The number of sites for inner stamens is influenced by the number and position of carpels. The flower completeness is related to the reduction /enrichment of flower structure, caused by changes in stamen number in the sites of stamen positioning (from 0 to 4), and in perianth whorl number (1 or 2). The number of stamens in any positioning site depends on the ratio of stamen primordia size to the available space of this site. The flower polymorphism in some genera covers several structures, different in merosity and completeness, whereas the same flower structure occurs in far related taxa of the family.

P0533. Androecial pattern in Didiereaceae and the relationships of the family

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According to molecular data Didiereaceae belong within the Caryophyllales to the monophyletic portulacaceous group: Two clades within paraphyletic Portulacaceae have given rise to the families Didiereaceae and Cactaceae, resp. (e.g. Applequist & Wallace 2001). Within the core Caryophyllales the number of stamens is variable (from many to few). Multistaminate androecia develop centrifugally, those with fewer stamens varying in number mostly are formed in one series on a small ring primordium. In

isomerous androecia the primordia may initiate directly at the floral axis. We present for the first time floral developmental data from members of all four genera of Didiereaceae: The 6-12 stamens arranged in one series are formed nearly simultaneously on a more or less prominent ring primordium. This ring primordium may be interpreted as homologous with a larger one found in the Dilleniaceae, sister to a broadly defined caryophyllid clade, judged from molecular data (Soltis et al. 2000). Further ontogenetical studies are required in the portulacaceous alliance contributing to current phylogenetic relationships in this still controversially discussed group.

P0534. The Scanning-Transferring; Dried and Fresh Plant Materials to the Computer with Isik Kutusu

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Abstract body

The **ISIK KUTUSU** for transferring pictures, images of motionless beings and objects to the computer, depending on the quality of the scanner.

This device provides remarkably good results in transferring three-dimensional images of motionless beings and objects to the computer with a qualitated imaging and printing when used with scanners. Our device also provides facility for the analysis of the images of various entities in a number of fields in digital environment with a magnitude up to 60 times bigger than the real object (6000 %). An equality has been formulated in order to give the approximate count of the real magnitude depending on the dpi and the zooming counts. The device is patented, up to 2007

<http://www.biyolojiegitim.yyu.edu.tr/proje/kutu/index.htm> But recently the description of device will be free for everybody on the web page. We will demonstrate; preparing, scanning and transferring plant materials to the computer with **ISIK KUTUSU**. Also the plant images of The Virtual Herbarium of Van Province is prepared with using **ISIK KUTUSU** will be introduce on web page <http://www.biyolojiegitim.yyu.edu.tr/flora/ingindex.htm>

P0535. Testing molecular-based sister group relationships: floral ontogeny and morphology of the *Antirrhinum*-containing subclade revisited

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Inflorescence and floral morphology and development were studied in *Aragoa* (Plantaginaceae) and related genera. Inflorescences of *Aragoa* and *Plantago* are lateral racemes. In *Aragoa*, the five sepals emerge from the abaxial to the adaxial side of the floral apex, but the mature calyx becomes actinomorphic. The four stamens arise simultaneously and before petal initiation. The four petals emerge unidirectionally but the corolla becomes actinomorphic. Aestivation is cochlear ascendent. The abaxial-adaxial inception of the calyx and corolla during early floral development in genera such as *Aragoa*, *Digitalis*, *Plantago* and *Veronica* may indicate that zygomorphy is ancestral in those genera. The tetramerous corolla, which is actinomorphic during middle and late development, and tetrandry are potential synapomorphies of the clade *Aragoa* + *Plantago*. Pentamery of the calyx and corolla appears to be plesiomorphic in the broader *Aragoa*-*Angelonia* clade. Inflorescence and floral development and morphology of *Aragoa* are essentially similar to those found in *Plantago*, which is consistent with the molecular-based sister group relationship between these genera.

P0536. Pollen morphological study of *Halimocnemis*, *Halanthium*, *Halocharis* and allied genera of tribe Salsoleae (Chenopodiaceae) in Iran.

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The pollen grains in Chenopodiaceae have been mostly considered to show no variation. Present studies were conducted to evaluate some characters including pore number and pollen diameter as distinctive features taxonomically. The comparative pollen morphology of the genera *Halanthium*, *Halimocnemis*, *Halocharis*, *Petrosimonia*, *Gamanthus* and *Climacoptera* belonging to a problematic complex and microscopic data by LM and SEM

are given. Pollen morphological studies of specimens clearly show distinctive position of *Petrosimonia*, with the highest pore number and the least pore number can be seen in *Halocharis*. The obvious different between pore number of the two species of *Gamanthus* suggests that they can be included in different genera.

Different communities of the species *Climacoptera turcomanica* showed different pore numbers distinctively which can be attributed to the possibility that this species can be divided to smaller taxa. The position of the genus *Climacoptera* in pollen pore diagram showed considerable distance from *Salsola* which confirms the acceptance of *Climacoptera* as an independent genus from *Salsola*.

P0537. Pre-meiotic RNA polymerases are sufficient for male but not female gametophyte development and fertilization in *Arabidopsis*

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Unlike animals, whose gametes are direct products of meiosis, plant meiotic products undergo additional mitoses, differentiation and development to produce multi-cellular haploid gametophytes (gamete plants) that contain the egg and sperm cells. The complex development and metabolic activities of plant haploid gametophytes is presumed to select against deleterious loss-of-function mutations that cannot be complemented in the haploid state. Consistent with this expectation, we show that female gametophytes defective for nuclear RNA polymerases I, II or III arrest early in development, indicating that the female gametophyte relies on transcriptional machinery encoded by its haploid genome. However, male gametophytes (pollen) bearing defective polymerase genes survive, grow and transmit the mutant genes to the next generation, showing that transcriptional machinery derived from the pre-meiotic pollen mother cell is sufficient for pollen development and fertilization.

P0538. Anther Development in *Melilotus indica*- some interesting features

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Melilotus indica is an annual fodder legume that flowers during winter months. Leaves are trifoliolate and flowers are yellow in colour arranged in a racemose manner. Pods are very small and one-seeded. The anther is tetrasporangiate. Anther wall development follows the dicotyledonous type. The mature anther wall is comprised of an outermost epidermis, edothecium, one middle layer and the inner most tapetal layer. The tapetum is of secretory type. At the sporogenous stage of microsporogenesis, tapetal cells become irregular and during late ontogeny a transverse septum is observed in the microsporangia. The septum is of tapetal origin that seems to be an adaptation for better nourishment of developing microspores. The mature pollen grain of *Melilotus indica* is tricolporate. The exine is reticulate, well differentiated into ectexine and endexine. The ectexine is distinguished in a tectum, bacula and foot-layer. Aggregation of pollen grains by means of small exinal connections has also been observed. Another interesting feature noticed during this study is the *in situ* germination of pollen grains.

P0539. A re-investigation of *Tetracera* pollen.

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Among 29 investigated dillenioid species only 6 neotropical *Tetracera* species have pollen dimorphism. We have studied 31 pollen specimens of bisexual and male flowers of 15 *Tetracera* species by LM and SEM. 6 of them are from Old World and have only 3-colporate pollen as other species in subfamily Tetraceroideae. 11 male flowers patterns and 2 bisexual flowers patterns (*T. tigarea*) have 3-colporate pollen grains, 11 bisexual flowers pollen patterns are cryptoporate. Exine is tectate, microperforate, endexine is very thin. Round pores are disposed in endexine. However, species have different number of pores, their exposition are not similar too. So, *T. parviflora* have 3-8-cryptoporate pollen grains with exposed pores. Endopores in *T. willdenowiana* pollen are hardly observed. We have found 1 specimen (*T. jamaicensis*) with a combination of 3-5-multicryptoporate, 3-porate and 3-short-coporate pollen. True

cryptoaperturate pollen is known also for some species of *Crameria* (Crameriaceae), but its exine and apertural structure is quite distinct. This phenomenon and its significance have been discussed.

P0540. Pollen morphology of the genus *Patrinia* Juss. (Valerianaceae) and its systematic implication

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Pollen of 17 taxa (13 species, 2 subspecies, 2 varieties; 40 specimens in total) of the genus *Patrinia* Juss. in Valerianaceae was examined with LM, SEM, and TEM. Pollen grains are mostly medium to large in size, tricolpate (rarely tetracolpate) with the characteristic halo surrounding the aperture, and oblate to prolate-spheroidal in shape.

In equatorial view, the pollen is elliptic to circular, and in polar view, they are mostly circular or rarely 3-lobed. The ornamentation of exine is echinate and the exine thickness is uniform throughout. Two major pollen types can be recognized on the basis of exine sculpturing patterns; - (1) Type I: exine is composed of only echinae (1.0-4.0 µm) without micro-echinae, and verrucae shallow (0.3-3.8 µm, in most taxa) or rarely absent (in *P. speciosa*). (2) Type II: exine is composed of massive echinae (4.0-8.0 µm) together with sparsely or densely micro-echinae, and prominent verrucae (4.0-7.4 µm, in *P. gibbosa*, *P. saniculifolia*, *P. triloba* var. *palmata* and *P. triloba* var. *triloba*). The earlier infrageneric classification systems of *Patrinia* are evaluated on the basis of the present data.

P0541. Pollen morphology of the Astragaleae (Leguminosae, Fabaceae) in Queretaro, Mexico

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The pollen of 13 genera and 40 species of the tribe Astragaleae (Leguminosae, subfamily Fabaceae) from the state of Queretaro is described and illustrated. Observations of pollen grains were carried out under light microscopy (ML) and scanning electron microscopy (SEM).

In the Astragaleae the occurrence of single grains is a constant feature. The principal differences in pollen among the various species involved size, shape, and sculpture pattern. The common type, occurring within the largest number of genera (8) is the tricolpate, reticulate single grain. *Coursetia*, *Cracca*, *Indigofera* and *Psoralea* have 3-colpate pollen grains with a microreticulate to tectate-perforate sexine. The pollen grains of *Brongniartia*, *Dalea*, *Harpalice* and *Marina* are 3-colpate with a microreticulate sexine. *Astragalus*, *Diphysa*, *Eysenhardtia*, *Piscidia* and *Tephrosia* have 3-colpate pollen grains with a microreticulate sexine. The pollen of the Astragaleae is rather heterogeneous; however must be emphasized the limited use of the apertural types when not considered in conjunction with the exine characters.

P0542. Pollen diversity in Mexican and Central American species of *Mimosa* (Leguminosae, Mimosoideae)

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About 115 species of *Mimosa* occur in Mexico and Central America, belonging to the five sections of the genus. Their pollen diversity includes that of the whole genus, with polyads, bitetrads, and tetrads of diverse shapes and sizes. Sect. *Mimadenia* groups mainly vines with 12-16-grained polyads (Southern Mexico to Brazil, Ecuador, Peru, and Bolivia). Sect. *Batocaulon* (Southern USA to Argentina), includes mainly shrubs and trees with bitetrads, which relate this section to the Old World species of *Mimosa*. Sect. *Calothamnus* (Guatemala to Argentina), *Habbasia* and *Mimosa* (both from Mexico to Argentina) share the pollen grains arranged in tetrads. Although these three tropical sections group trees and shrubs, the latter includes most herbaceous species with the smallest tetrahedral tetrads. Pollen diversity contributes important systematic characters for understanding the relationship among infrageneric taxa of *Mimosa*. Bitetrads found in a species of sect. *Mimadenia*, and rarely observed in sect. *Mimosa*, suggest a link with members of sect. *Batocaulon*. Furthermore, polyads in sect. *Mimadenia* support the relationship between *Mimosa* and *Piptadenia*.

P0543. Pollen tube behaviour of the "feronia" mutant in *Arabidopsis thaliana*

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Accomplishment of fertilization in higher plants requires the direct interaction of male gametophytes, i.e. pollen and pollen tube, with the female reproductive organs including the female gametophyte (embryo sac). In wild-type plants the pollen tube enters the degenerated synergid, arrests and releases the sperm cells, which are targeted to the egg and central cell, respectively. The female gametophytic mutant *feronia*, shows a failure in the direct interaction of pollen tube and embryo sac. In this mutant pollen tubes continue to grow inside the degenerated synergid and get tangled up within the micropylar tip of the female gametophyte. The study of synergid marker expression and the ultra-structural analysis of female gametophytes suggest that the synergids are normally specified and differentiated in mutant embryo sacs. Therefore the defect in *feronia* is likely due to the disruption of a direct interaction between the male and female gametophyte. We will report on the molecular isolation of FERONIA, which provides new insights into this novel signaling pathway in flowering plants

P0544. Angiosperm micropyle selects a single pollen grain from many for fertilization

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In contrast to gymnosperms, whose naked ovules usually receive many pollen grains, angiosperm ovules enclosed in a pistil receive only a single pollen (tube) for fertilization. The pollen tube is guided by and reaches the embryo sac through tissue of the pistil. To discuss where and how a pollen tube is guided and selected, we show that in *Fagus japonica*, where ovules are immature at pollination, growth of the pollen tube stops at the ovarian locule and micropyle, then grows intermittently in three steps for about 5 weeks. In the 1st step, the pollen tube proceeds irrespective of mature sporophytic tissue in the ovule and differentiation of the embryo sac; step 2 proceeds after formation of ovular sporophytic tissue and differentiation of the embryo sac; step 3 occurs after maturation of the embryo sac. Pollen tubes are guided to the micropyle by sporophytic tissue in the pistil, and from the micropyle to the embryo sac by the embryo sac itself. Besides, the micropyle selects a single pollen grain from among what have survived after competition in the pistil. The selection of a single pollen grain by the micropyle may have contributed to the explosive evolution of angiosperms.

P0545. Investigation of the reproductive potential of Serbian spruce - correlations of microstrobiles, pollen and seed properties

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Endemic and relic tree species, Serbian spruce (*Picea omorica* Panc./Purkyne), is especially significant for the region of the Balkan Peninsula. The study of the genetic potential of the reproductive material of this conifer (microstrobiles, pollen and seed) contributes to the provision and availability of good-quality seed and plants. The observed material originated from 25 test trees in forest plantation at the locality Bela Zemlja near Užice in Serbia and Montenegro. Morphological and physiological properties of microstrobiles, pollen and seed (dimension, vitality, germination percentage) on the level of the population and on the level of the individual tree were analysed. Results have shown the diversity structure and the potential of this population. This potential is important for the further breeding of Serbian spruce and for application in forestry and in environmental protection.

P0546. Pollen studies in the subfamily Bromelioideae (Bromeliaceae)

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Bromelioideae is the most diverse subfamily of Bromeliaceae, mainly distributed in Brazil. It is considered an euripolinic group and has some genus with a complex circumscription. In order to support a better classification in Bromelioideae were studied the pollen morphology of 30 species in the genera: *Acanthostachys* (1), *Aechmea* (9), *Billbergia* (2), *Edmundoa* (2), *Fernesea* (1),

Nidularium (12), *Quesnelia* (2), and *Wittrockia* (1), which 14 species are analysed for the first time. The material was submitted to lactic acetolize, measured and applied statistical treatments with graphic representation, described and photomicrographed under light and SEM. The pollen grains vary from medium to large size, elliptical, ovulate or subrectangular amb, porate or colpate, exine reticulate or rugulate. The preliminary results separate some species through morphologic types as a size, form and aperture that were considered important diagnostics characters for some genus and species.

P0547. The pollen morphology of the wild *Primula* L. (Primulaceae) species in Turkey

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8 species (*P. davisii* W. W. Sm., *P. vulgaris* Huds., *P. veris* L., *P. elatior* (L.) Hill., *P. megaseifolia* Boiss & Bal. Ex Boiss, *P. longipes* Freyn & Sint., *P. auriculata* Lam. & *P. algida* Adams) and 7 subspecies (*P. vulgaris* Huds. subsp. *vulgaris*, *P. vulgaris* Huds. subsp. *sibthorpii* (Hoffm.) Smith & Forr., *P. veris* L. subsp. *columnae* (Ten.) Lüdi., *P. veris* L. subsp. *macrocalyx* (Bunge) Ludi., *P. elatior* (L.) Hill. subsp. *meyeri* (Rupr.) Val. & Lam., *P. elatior* (L.) Hill. subsp. *pallasii* (Lehm.) Smith & Farr. and *P. elatior* (L.) Hill. subsp. *pseudoelatior*) of *Primula* L. (Primulaceae) were studied palynologically by light and scanning electron microscopy. The pollen is tricolpate, triparasyncolpate, polycolpate or spiraperture with reticulate sculpturing. Aperture of *Primula* taxa are changing from polycolpate to triparasyncolpate-tetracolpate, from primitive to progressive. Two pollen variants are distinguishable the short-styled species have bigger pollen and thicker exine and intine than the long-styled species.

P0548. Palynology of the Anacardiaceae: Tribe Spondieae

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The pollen morphology of the Anacardiaceae is studied to contribute to the systematic position of the family. At present 32 species belonging to 17 genera of the tribe Spondieae are investigated by light and scanning electron microscopy. The Spondieae pollen is characterized by 3-colporate (rarely 4-colporate) isopolar and monads. Their size and the P/E ratio averages between 21.7 to 82.1 and 0.79 to 1.53 µm respectively. Pollen grains are prolatespheroidal, subprolate and prolate and they show a circular outline in polar view. The pori are mostly longitudinal, rarely longitudinal and vary in size. The exine thickness varies between 1.6 to 3.4 µm. The sexine is generally thicker than the nexine. Various sculpturing of the tectum is found from psilate-perforate, striate-reticulate, striate-rugulate and striate. Seven pollen types are recognized and three groups of preliminary phylogenetic morphological trends emerge from this study. These present pollen morphological results support the existing taxonomic investigations by macro morphology and molecular studies.

P0549. Microsporogenesis in *Thalassia testudinum* Banks ex Köning

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Thalassia testudinum is a marine angiosperm from the (sub-) tropical Western Atlantic. The species is dioecious. Present work concerns the development of male reproductive structures (microgametogenesis) and the microstructure of the pollen grains. Male floral buds at different stages of development were collected during late-winter and spring of 2001 and 2002. Part of this material was fixed in FAA and processed for paraffin inclusion, and the microtome cut sections were stained with the safranin-fast green technique. The rest of material was fixed in glutaraldehyde-paraformaldehyde-s-collidine buffer, included in LRwhite and cut with a glass knife to obtain 1 µm thick sections in ultramicrotome. These preparations served to present a first accurate description of *Thalassia testudinum* male flower development. The wall of the anther was of monocot type, the microsporogenesis is successive with linear isobilateral and T-shape tetrads and the tapetum is atypical periplamodial. The mature pollen grains were round with a thin exine layer with microequinate ornamentation.

P0550. Pollen morphology of *Acalypha* (Euphorbiaceae) in the Malaysian Region

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In the Malaysian region, about 30 species of *Acalypha* are recognized out of the 450 species known worldwide. Pollen diversity in *Acalypha* was previously examined in 73 species representing most of the taxonomic diversity in the genus using LM, SEM, and TEM. Results show that *Acalypha* pollen has two main types of exine ornamentation, one having a microrugulate to rugulate ornamentation with suprategal elements distributed on the margins of the muri, and the other having a nearly areolate ornamentation with suprategal elements scattered all over the pollen surface. These previous observations show that *Acalypha* pollen is not homogenous as described in earlier studies. We report here on the pollen morphology of *Acalypha* in Malesia. Our preliminary findings based on scanning electron microscopy include two new ornamentation types, one having areolate ornamentation with fossulae and scabrae scattered on the areolae; whereas the other exhibits a microrugulate ornamentation with fossulae on the murus. These pollen characters show a possible correlation with macromorphological characters and are potentially significant for phylogenetic analysis.

P0551. Comparative pollen biology of the tropical legume *Indigofera suffruticosa* occurring in Brazilian sea land and savanna disturbed areas

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Micro morphology, ultra structure and cytochemistry of the pollen grain have been compared with individuals of *Indigofera suffruticosa* from Brazilian sea land (seashore) and savanna (countryside) disturbed areas. Pollen variations have occurred among populations from both vegetal formations. In the savanna, viability and pollen diameter are significantly higher than those found in the sea land. Sexine ornamentation varies from gemmate to perforate. In the vegetative cell cytoplasm mitochondriae are more ovate with well developed and contrasted crests in the savanna; in the sea land mitochondriae are less contrasted with dumbbell-shaped, less evident crests. Pollen grains from the savanna have shown more positive reactions to proteins, lipids and nucleic acids. Sea land individuals do not present abnormalities in pollen grains although there is less pollen viability. It is probable that these individuals have adopted different reproductive strategies for better environment conditions. Mitochondriae, for instance, possibly present different functioning related to their crests, types and quantities of proteins observed on membranes (FAPESP).

P0552. Pollen morphology of the perennial species of genus *Lathyrus* L. (Fabaceae) in Bulgaria

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The pollen morphology of all 19 perennial species from genus *Lathyrus* L. (Fabaceae) distributed in Bulgaria was studied with LO microscope and SEM as a part of biosystematical research. The results reveal that the pollen grains are 3-zonocolporate, elliptical or rectangular in equatorial view, circular to triangular-obtuse-convex in polar view, medium to large in size (P x E = 26.9-66.4 x 20.5-53.7 µm). The aperture system is composed of straight ectocolpi with thick costae along the margins and large, circular or slightly elliptical endopori. The thickness of the exine is 0.6-1.8 µm, the sexine usually as thick as the nexine. The infratectum is composed of rods and rarely of granules. The ornamentation in mesocolpium is predominantly suprareticulate with small lumina with wide muri. The apocolpium is usually psilate or with scattered puncta. The palynotaxonomical characters studied assign the pollen grains to 3 major pollen types: *subprolate* - sect. *Orobis*, sect. *Lathyrus*; *prolate* - sect. *Pratensis*, sect. *Lathyrystylis* (*L. digitatus*, *L. pannonicus*) and *prolate spheroidal* - sect. *Lathyrystylis* (*L. pallescens*, *L. panicii*).

P0553. Pollen Morphology of Orobanchaceae Tribe Rhinanthaeae in Iran

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According to molecular data, tribes Rhinanthaeae (parasite) and Gratiolaeae (non-parasite), formerly belonging to Scrophulariaceae, have been considered to be members of Orobanchaceae. In this study, light microscopy and scanning electron microscopy, were used to examine the pollen of 11 species of 8 genera the tribe *Rhinanthaeae* plus *Lindenbergia indica* from *Gratiolaeae*. The resulting data showed the pollen of Rhinanthaeae is similar to that of Orobanchaceae s. str. as previously indicated, and thus they are closely related. The grains were typically isopolar tricolpate, radially symmetrical, more or less subprolate. Exine sculpturing is reticulate (*Pedicularis caucasica*, *Rhinanthus vernalis*, *Odontites verna*, and *Lindenbergia indica*), retipilate (*Euphrasia pectinata*, *E. juzepczukii*, *Bungea trifida*, and *Parentucellia latifolia*), perforate-rugulate (*Odontites aucheri*), retirugulate (*Bellardia trixago*), and scabrate-verrucate (*Rhynchosorys maxima* and *R. kurdica*). It seems that reticulate exine sculpturing is plesiomorphic among taxa studied.

Keywords: Orobanchaceae, Palynology, Rhinanthaeae

P0554. Echinat Compositae pollen from Middle Russia

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150 species of echinate Compositae pollen divided in 12 pollen-types were studied with special attention to the sculpture. The group with rather small (<1µm) spines includes 6 pollen-types: 1) Echinops - with large pollen grains and two columella layers; 2) Scabiosa - large pollen grains with cavities; 3) Xanthium with smaller pollen grains and short colpi; 4) Artemisia - rather small spherical p.g. with long colpi and circle ora; 5) Jacea - rather small elliptic pollen grains with long colpi and oval ora; 6) Cyanus - with verrucate sculpture. The group with big (>2,0 µm) spines was divided into 6 pollen-types: 1) Ambrosia - rather small p.g. with numerous spines; 2) Serratula - rather large p.g. with numerous spines; 3) Tussilago - spines rather high with sharp peaks distanced each other; 3) Bidens - spines have elongated peaks on hemisphere; 4) Helianthus - spines are narrow conic; 5) Cirsium - spines are connected with low bridges; 6) Senecio - spines are broad conic.

P0555. Evolution trends of Asterales sporoderm ultrastructure

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Five ultrastructural types of sporoderm are known among Compositae: Chuquiraga, Nassauvia, Anthemis, Helianthus, Cichorium. Pollen grains of another Asterales (Goodeniaceae, Calyceraceae, Brunoniaceae) belong to Anthemis type. It is characterized by large columellae based on foot layer. The outer parts of columellae split and form reticulate infratectum. The outermost parts of columellae are straight and make comb-like appendixes connected with tectum. We suppose, this type is initial for Compositae, through intermediate forms it is connected with Helianthus, Cichorium, Nassauvia types. The Chuquiraga type (subfamily Barnadesioideae) is isolated and most original. Pollen grains of this type have high and thin columellae with lateral appendixes. Appendixes alternate with electron dense granules and form the regular lines. Foot layer is discovered only around apertures. The analysis of Compositae sporoderm structure allows to reveal some evolutionary trends: increase in sporoderm thickness by prolongation and bifurcation of columellae; differentiation of nonapertural parts of ectexine; development of cavities and foraminae.

P0556. A Study on Pollen Morphology and Viability of Summer Squash (*Cucurbita pepo* L.)

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In this study some pollen grain characteristics (pollen link type, symmetry conditions, pollen size, pore size and pore number) were determined with SEM and viability were evaluated by using staining methods with safranin (Essad 1962), IKI (MacLean and Cook 1941) and Triphenyl Tetrazolium Chlorid (TTC) (Stanley and Linskens 1974), in vitro germination test. Pollen grains were taken

from some inbred lines in I₆ for summer squash (*Cucurbita pepo* L.).

Experiment results showed summer squash pollen's link type is monad, symmetry conditions is isopolar, sizes are 126.3 µ x 117.5 µ, pore sizes are 27.45 µ x 28.86 µ, pore numbers are 11-13. Viability of pollen of summer squash the 28 % pollen viability. In staining methods, pollen viability percentages were found higher than in vitro records. This results showed that staining methods give a rough estimation of pollen viability. Because pollen grains yet to mature and/or unviable may also get stained.

P0557. Earliest records of the angiospermous pollen in Razdolnenskaya basin (the South Primorye)

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The angiosperm sequence was studied in the continuous Barremian to the lower Cenomanian of Razdolnenskaya basin. For the first time the angiospermous pollen appeared in the early Albian. These are *Clavatipollenites incisus* Chlon., *C. sp.*, *Tricolpites sp.*, *Retitricolpites sp.*

Diversity of angiosperms increased in the middle Albian. *Clavatipollenites hughesii* Coup., *Retitricolpites vulgaris* Pierce, *Asteropollis asteroides* Hedl. et Norr. appeared.

Tricolpites micromunus (Groot et Penny) Singh, *Fraxinipollenites variabilis* Stanl., *Rouseia delicipollis* Sriv., *Polyporites clarus* N. Mtch. appeared in the late Albian, and *Tripoporipollenites sp.*, *Trocolporipollenites sp.* - in the early Cenomanian.

Thus, the first rare angiosperms appeared in the early Albian and their participation and diversity in palynoflora become abundant in the early Cenomanian.

The study was supported by Presidium RAS and FEB RAS (grants 05-1-P12-022, 05-1-P25-078).

P0558. Pollen morphological study of *Halimocnemis*, *Halanthium*, *Halocharis* and allied genera of tribe Salsoleae (*Chenopodiaceae*) in Iran.

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The pollen grains in Chenopodiaceae have been mostly considered to show no variation. Present studies were conducted to evaluate some characters including pore number and pollen diameter as distinctive features taxonomically. The comparative pollen morphology of the genera *Halanthium*, *Halimocnemis*, *Halocharis*, *Petrosimonia*, *Gamanthus* and *Climacoptera* belonging to a problematic complex and microscopic data by LM and SEM are given. Pollen morphological studies of specimens clearly show distinctive position of *Petrosimonia*, with the highest pore number and the least pore number can be seen in *Halocharis*. The obvious difference between pore number of the two species of *Gamanthus* suggests that they can be included in different genera.

Different communities of the species *Climacoptera turcomanica* showed different pore numbers distinctively which can be attributed to the possibility that this species can be divided to smaller taxa. The position of the genus *Climacoptera* in pollen pore diagram showed considerable distance from *Salsola* which confirms the acceptance of *Climacoptera* as an independent genus from *Salsola*.

P0559. Forensic Pollen Evidence from the Clothes by the Tape Adhesive Method

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Collection and identification of pollen is becoming important in forensic applications. Many criminal cases had reported to link suspects to crime scene by analysis of pollen. Several methods had been used in the pollen collection and analysis, but they are expensive and tedious. Therefore, it is important to develop a simple method to collect pollens from clothes. We tried to recover pollen from clothing surface by using sticky tapes method. Different kind of commercial tapes, textiles and different species of pollen were included in the testing. Result showed that tape D and weave textile S had better adhesion of more pollen than other weave textile. We wear clothes that were made from textile S to collect pollen from 26 different areas mostly in northern part of Taiwan and then used tape D to recover them from different part of

body. From this result, we concluded that tape adhesive method is suitable to recover pollen from clothes. We suggested that tape adhesive method could be one of methods for collecting pollen from clothes of suspects. It is simpler, faster and more inexpensive than other methods.

P0560. Nitric oxide (NO) modulates growth and development in *Micrasterias denticulata*

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Nitric oxide (NO), a key molecule in intracellular signalling, may modulate developmental processes as well as host defense mechanisms. We investigate the effect of NO on developmental stages of the unicellular green alga *Micrasterias denticulata* by use of S-nitroso-N-acetylpenicillamine (SNAP), a common NO-donor, and the donor molecule (N-acetylpenicillamine; NAP) alone. NAP-treated cells show no difference in development when compared to control cells, whereas cells incubated with SNAP are markedly retarded in cell growth. Investigations at the electron microscopic level reveal that the retarded cells lack a secondary cell wall. Moreover, golgi stacks are slightly involute and dictyosomal productivity is reduced. In NAP-treated and control cells both secondary cell wall formation and dictyosome morphology appear normal. Therefore, we conclude that NO either inhibits or retards secondary cell wall formation in *Micrasterias* via altering dictyosomal activity.

P0561. Study of the plant-specific myosin XI class

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The correct functioning of plant cells, hence of plant growth and morphogenesis, depends on several processes based on the actin cytoskeleton. Myosins are the motor proteins responsible for the actin-based motility. Phylogenetic analysis revealed that the plant myosin family has evolved in a totally divergent way among eukaryotes, suggesting that plants have adapted differently to fulfill their distinctive lifestyle. Although the currently known myosins have been grouped into 18 phylogenetic classes, all plant myosins seem to fall into only three unique and plant-specific classes - class VII, XI and XIII. In the last decade, a great deal of attention has been given to the study of the function and regulation of the different myosin proteins in animal and yeast cells. However, little is known about the function of specific myosins in plants. We focused our study on class XI of plant myosins, a class structurally similar to myosin V from animals and yeasts, and thought to be involved in vesicle trafficking in plants. The data presented will be discussed on the light of previous ones.

P0562. Acanthochlamyaceae

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Only one sp., *Acanthochlamys bracteata* P. C. Kao, subalpine xerophytic valley zone of W Sichuan and SE Tibet, SW China.

Acanthochlamys bracteata is restricted to the Hengduan Mountains at the SE margin of the Kang-Zang Plateau of SW China (Xiangcheng, Daocheng, Daofu of W Sichuan to Zhag'yab of Tibet), where it occurs in the subalpine aciculignose shrub-meadow region at an altitude of 2700-3500m.

The sieve-element plastids of *Acanthochlamys* differ from those of the Velloziaceae by their lack of loosely-packed crystals and from the Amaryllidaceae and all families of the order Pandanales by their small sizes.

Among all families compared here with *Acanthochlamys*, the absence of cell wall-bound ferulate is shared only with the Velloziaceae. Thus the morphological data point in the same direction as the molecular analysis, although the relationship between *Acanthochlamys* and the Velloziaceae may not be very close.

In cladistic analyses of the comparative rbcL data a 100% support for the distinction of *Acanthochlamys* from the Velloziaceae as well as from the other families of the order was found.

P0563. Differentiation of leaf anatomy of the genera *Dracaena* L. and *Sansevieria* Thunb. (Dracaenaceae).

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The family Dracaenaceae was described by Salisbury in 1866. In recent literature it is said to include two genera: *Sansevieria* Thunb. and *Dracaena* L. or only one - *Dracaena*. Thorough anatomical leaf studies were reported only for *Sansevieria*. Studies under light microscope, SEM and confocal laser scanning microscope on living and dry, dissected material of 20 taxa of *Dracaena* and 10 taxa of *Sansevieria* have indicated, that their leaves show significant differences in sculpture and anatomical structures, which are genus specific. Both qualitative and quantitative characters were taken into consideration. Main dissimilarities are visible in: presence or lack of water tissue, type of stomata, presence or lack of cuticular band around stomata, distribution of stomata and distribution of vascular tissue. Because of specific intermediate anatomical characters of the leaf lamina, *D. cinnabari* Balf. f. - an Soqotra endemite and *D. ellenbeckiana* Engl., should be regarded as separate cases within this family.

P0564. The occurrence of tubers in *Noteroclada confluens* Taylor ex Hook. & Wilson

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Noteroclada confluens is a monotypic, simple thalloid liverwort that grows in moist, montane habitats from Mexico to Tierra del Fuego and southeast Brazil. Surprisingly, we have discovered that 24.3% of 296 specimens studied from across its range produce tubers. These usually arise from branch initials on main stems, but may also develop from main shoot or long branch apices. Mature sporophytes and tubers can be present on the same plant. In some populations, the mature tubers are cylindrical, 0.42-2.52 x 0.23-1.14 mm, but in others even at maturity the tubers are spheroidal, 0.40-1.52 mm in diameter. Populations with cylindrical forms are most common in southern Brazil, while those with the spheroidal forms are more widespread. SEM and paraffin sectioning techniques were used to study the structure and development of these tubers. In young tubers the apical cell is replaced by a flattened meristem; as the tubers mature, both the surrounding leaves and the epidermal cells become thick-walled and the interior cells are packed with large starch grains. It is speculated that the tubers function primarily in nutrient storage and only secondarily as asexual propagules.

P0565. Molecular characterization of a short grain mutant isolated by rice activation tagging

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We have generated 13,000 activation-tagging lines of rice. Several mutants including lesion mimic, stripe, dwarf, and short grain were obtained in the activation-tagging lines. By the analyses of a lesion mimic mutant (*Lmm1*), we have already shown that the overexpression of a gene showing sequence similarity to an acyltransferase caused lesion mimic phenotype.

In this research, we analyzed the *Short grain 1* (*Sg1*) mutant. When the T-DNA insertion is heterozygous, the grain is small, and when it is homozygous, the grain is very small, indicating that the *Sg1* phenotype is linked to the T-DNA insertion. The *Sg1* plant also shows semi-dwarf phenotype and the phenotype is restored by the addition of gibberellin. A full-length cDNA lies 1.4 kb downstream of the inserted T-DNA and it expresses mainly in the young panicle in wild type. The deduced protein shows no homology with any known proteins. These results strongly suggest that transcriptional activation of this novel gene causes the *Sg1* phenotype.

This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Rice Genome Project MP-1203).

P0566. Inhibition of *FLORICAULA/LEAFY* Orthologue Expression with RNA Interference in *Chenopodium rubrum* L.

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The homologous transcription factors *FLORICAULA* of *Antirrhinum* and *LEAFY* of *Arabidopsis* share conserved roles in floral meristem identity and floral patterning. We have identified the *C. rubrum* *FLO/LFY* orthologue (*CrFL*), that showed changed expression during flower induction. To determine the *FLO/LFY* role in the floral development we suppressed its expression using the RNAi method. For this purpose, we constructed the binary vector pFGC5941 containing a 35S-promoter sequence followed by a cDNA fragment of the *CrFL* in sense orientation, an intron of the *CHSA*-gene and a second copy of the cDNA fragment of *CrFL* in antisense orientation. To introduce this T-DNA construct into *C. rubrum* we established a protocol for efficient *Agrobacterium*-mediated transformation through floral dip method. Herbicide selection and PCR analysis showed a very high transformation rate while using the optimized protocol. The phenotypes of transformed plants were analyzed.

P0567. On Specificity of Functional Organization and Individual Development of Modular Objects

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Functional specificity determines many peculiarities of individual development, ecology and evolution of modular objects. Principal difference of modular organization is that development function and regulation are connected with changes of object macrostructure. Dynamic macrostructure determines the instability of borders of a system, high degree of interaction of main elements of organization, complexity in their splitting and independent investigation. The description of macromorphological structure includes the analysis of its dynamic and functional aspects. It makes very actual the development of theoretical model of modular object morphology. Function and development of modular objects are similar to those in population systems. Analysis of function, individual development and structural evolution of modular objects compared with unitary organism shows lower degree of integrity, development and transformation of the latter. Numerous analogies between modular and unitary systems allow to use modular organization as a model to clarify the ways of realization of general tendencies of organization transformation.

P0568. Regulation of nitrogen metabolism by amino compounds and cytokinins in poplar

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In the present study the effects of additional supply of glutamine or cytokinin on nitrate net uptake and expression of genes involved in N reduction were addressed in young poplar trees. Incubation of poplar roots in media containing either Gln or cytokinin (zeatin riboside, ZR) resulted in a decrease of N net uptake. Plants fed Gln revealed significant enrichment in Gln and other amino compounds of different tissues. Exogenous supply of t-ZR induced variable, incubation time-dependent expansion of the pool of Z-type CKs, while the levels of amino compounds accumulated in the tissues, were comparable to controls. Expression level of nitrate reductase in fine roots was higher as a result of the cytokinin treatment. Gln fed plants showed reduction of NR expression. Expression of the cytosolic glutamine synthetase was changed or not influenced by the treatments, dependent on the time of root incubation in the media. From these results, regulation of N-uptake at the whole plant level is discussed. The presented work was performed in collaboration with the Poplar Research Group Germany.

P0569. Leaf anatomy and its relation to the ecophysiology of 30 weed species of different seasonal performances

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The present work demonstrates the leaf anatomy of 30 weed species representing winter, summer and all-year weeds. The species were collected from the field crops of Beni-Suef governorate, Egypt. Some anatomical differences were observed between the leaves of the different weed groups. Such differences may be related to the seasonal variations in climate. Compared to winter weeds, the investigated summer weeds possess higher stomatal and trichome densities as well as higher leaf specific conductivity (*LSC*). Moreover, summer weeds exhibit lower calculated hydraulic conductance (*Kh*) of tracheary elements supplying the transpiring areas, laminal area and stomatal spacing than winter weeds. For all-year weeds with either winter or summer affinity, the stomatal density is generally higher in summer than in winter season. In general, the laminal area, *Kh*, and number of tracheary elements of petiole or petiolule are significantly higher in winter than in summer season for the studied all-year weeds with winter affinity. On the other hand, the all-year weeds with summer affinity show an opposite behaviour.

P0570. Physiological Phenomena in a Range of Seeds Drawn From the New Zealand Flora

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Germination behaviour of seeds of New Zealand's unique flora is imperfectly known. Dormancy status and summary germination phenomenology are reported for a range of species drawn from the diverse families of angiosperm seed plants including: *Myrsine australis* (Myrsinaceae), *Coprosma robusta* (Rubiaceae), *Sophora prostrata* (Fabaceae), *Muehlenbeckia australis* (Polygonaceae), *Leptospermum scoparium* (Myrtaceae), *Hebe stricta* (Scrophulariaceae), *Myosotidium hortensia* (Boraginaceae), *Euphorbia glauca* (Euphorbiaceae), *Cyperus ustulatus* (Cyperaceae), *Carex trifida* (Cyperaceae), *Phormium tenax* (Agavaceae), *Vitex lucens* (Verbenaceae), *Laurelia novae-zelandiae* (Monimiaceae), *Pachystegia insignis* (Asteraceae), *Dacrycarpus dacrydioides* (Podocarpaceae) and *Rhopalostylis sapida* (Arecaceae). Population heterogeneity, germination kinetics, dormancy mechanisms where present, seed structure, fruit-seed relationships and dispersal modes are discussed.

P0571. Influence of osmotic potential and imbibition period on priming efficiency in folk silk tree seeds

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The positive results of priming, mainly in agricultural crops, confirm its potential to improve seed and seedling vigor, but there is a lack of information about the results in Brazilian wood native species, due the wide flora biodiversity. *Chorisia speciosa* (St.Hil) (Bombacaceae) is a deciduous wood species, occurring in several Brazilian states. It is cultivated in gardens, squares, city avenues, and indicated for mix plantations in disturbed areas. Intact seeds were primed in distilled water and PEG solutions (-0.2; -0.4 and -0.8 MPa) during 24, 48 and 72 hours at 27°C. The seeds were washed, dried until reach the initial weight and after exposed to thermal stress at 50°C during 24, 48 and 72 hours. It was registered a decrease in the rate and germination percentage, seedling emergence and dry mass, as the thermal stress intensity increased. Priming did not overcome this effect. It is possible to conclude that PEG solutions decrease seed vigor, detected by conductivity and germination tests, but no rule could be established in relation to solution concentration, for all the tests. There was a negative effect on seed and seedling vigor as the imbibition time increased.

P0572. Response of symbiotic and nonsymbiotic *Chlorella* to norflurazon

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The effect of norflurazon on endosymbiotic *Chlorella vulgaris* Beij. [K&H, 1992] from green hydra (*Hydra viridissima* Pallas, 1766) and

asymbiotic *Chlorella kessleri* Fott et Novak. [K&H, 1992] was researched. In this work we observed the difference in response to norflurazon between high-related algae in symbiosis and out of it. Norflurazon causes the "bleaching effect" upon the newly developed chloroplasts. Organisms were treated with aqueous solutions of norflurazon (2×10^{-4} , 2×10^{-5} , 2×10^{-6} , 2×10^{-7} and 2×10^{-8} mol/L) in glass dishes, and in tubes on deep stock agar in the laboratory conditions. Depending on the concentration, norflurazon caused cytological and ultrastructural changes that were observed with binocular light microscope and TEM. Of special significance were the antichloroplastal and antimitochondrial effect. Newly developed endosymbiotic algae in green hydra were not bleached. The "bleaching effect" was present only in algae out of symbiosis, with deleterious effect upon algal thylakoidal system. Mitochondria were swollen and torn apart. Endosymbiotic alga was less damaged at all than alga out of symbiosis.

P0573. Structural and functional changing in generative organs of Siberian coniferous species under ecological stress and there biotechnology in culture in vitro

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Unfavorable ecological factors influences on generative organs of Coniferous species. Under the global climate warming the absent of organic dormancy in male generative buds of Siberian larch and Scotch pine in winter is observed. An early meiosis, a great number of abnormalities in the meiotic division process as well as gametophyte formation result in formation of sterile pollen, also in a weak pollination and, as a result, in a weak (or in absolute absence) cone and seed yield. Pollen sterility increases in regions with a high technogenic pollution. To overcome sterilizing and breeding improved tree genotypes of conifers species the studies of reproductive processes in culture in vitro were carried out. The cultivation of microsporophylles of Siberian larch and Scotch pine in the autumn-winter period resulted in formation androgenous callus and embryoids. Cultivation of immature zygotic embryos at the early differentiation stage promoted getting somatic embryoids and plantlets of Siberian larch and Siberian pine. Thus, androgenic cultures and somatic embryogenesis open the way for obtaining the high-value productive Siberian coniferous trees.

P0574. Seed germination of Dalbergia miscolobium Benth. a savannah tree species from Brazil.

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Dalbergia miscolobium Benth. (Papilionaceae) is a common tree in Brazil Cerrado biome. We describe the germinative behavior of *D. miscolobium* seeds, under 12 different isothermal temperatures (10, 15, 20, 24, 27, 30, 34, 38, 40, 44, 48 and 50°C), water stress simulated with PEG 6000 solutions (0, -0.2, -0.3, -0.4, -0.6, -0.8, -1.0, -1.2 MPa) and thermal stress (60 and 70°C during 24, 48 and 72 hours) and the imbibition water uptake. The phase 1 of imbibition ends around 28h and the phase 2 extend nearly to 116 hours of the germination process. The optimal temperature for the germination is around 30°C and the optimal range of for germination were between 20 and 40°C. The cardinal temperatures for germination were between 10, 15°C (lower limit) and 48, 50°C (higher limit). The limit of germination under water stress was between -1.0MPa and -1.2MPa and seed viability decreases with the increase of thermal stress. We conclude that *D. miscolobium* is a eurithermic, thermal and water stress tolerant species. Such large tolerance limits can partially explain the biogeography and the wide area where this species is nowadays scattered.

P0575. Sudden shifts in stress coping strategies along stress gradients: A case study on shoot stiffness in salt marshes

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We hypothesized that along stress gradients, sudden shifts between alternative stress coping strategies may occur. This was tested in vegetation in tidal habitats where hydrodynamic forces increase with decreasing elevation towards the sea. Species growing at the lowest elevations can survive the high forces there

either by producing very flexible stems and go with the flow or tolerate it with very rigid and strong stems. We characterized the mechanical properties of the shoot (length, Diameter, Young's modulus [E], breaking stress [σ_b] and flexural stiffness) for ten species, and integrated these into the overall flexibility and strength of the entire shoot. Overall shoot strength and flexibility were found to be either very low or very high in species around the sea border and intermediate for species at higher elevations. Differences in overall flexibility and strength were mostly correlated with variation in diameter and height and less with E and σ_b . These results confirm our hypothesis and were integrated into a general conceptual model.

P0576. Investigation of some physiological responses of two *Aeluropus* species to water deficit and salinity

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In the present investigation, stomatal resistance, soluble sugars, proline content and peroxidase enzyme activities of two *Aeluropus* species (*Aeluropus littoralis* and *A. logopoides*) was studied in response to water deficit and salt stress. A completely randomized factorial design with four replications and different combination of four levels of water deficit (-0/3, -0/5, -10 and -15 bar), four levels of salinity (0, 20, 25 and 30 dSm⁻¹) and two species of *Aeluropus* was used under glasshouse conditions. Water deficit was induced by weight method on the basis of the amount of water lost each day. Salt stress was made by addition of sodium chloride to the initial water applied. One month after stresses were imposed, two-weekly interval stomatal resistance recorded, and after two months soluble sugars and proline content, peroxidase enzyme activities were measured. Results showed that by increasing water and salt stress, stomatal resistance was first increased and then started to decrease. Soluble sugars and proline content and peroxidase activities were also increased in response to both types of stresses.

P0577. Boron remobilization in *Olea europaea* L. is regulated by external boron supply

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Boron (B) is an essential micronutrient for higher plants. Although B remobilization is restricted in many plant species, sugar-alcohol-B complexes may be translocated from developed (source) to developing (sink) organs in species that export sugar-alcohol photoassimilates in the phloem sap. In the present study, physiological factors affecting the process of B remobilization in *Olea europaea* were studied under various (adequate to deficient) levels of B supply. It was found that the concentration of B in the cell sap of source-leaf tissues is the main factor affecting the amount of B loaded in the phloem sap. Therefore, under adequate supply, higher amounts of B were remobilized, compared to those under deficient conditions. However, B deficiency dramatically increased the relative amount of B (expressed as the ratio of phloem sap B / cell sap B of the corresponding leaves) that was remobilized from source leaves. In these leaves, phloem sap mannitol concentration was 2-fold higher under deficient compared to adequate conditions. It is suggested that, in this plant species, mannitol is involved in the regulation of B remobilization as a response to B deficiency.

P0578. Phenolic content from different cultivars of sugarcane juices in response to smut, leaf scald and yellow leaf syndrome

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Sugarcane juices contain different phenolic acids which can appear both as free and conjugated forms. The aim of the present study was to compare the variation of phenolic concentration in response to different sugarcane diseases by using healthy (as control) and inoculated plants.

To study smut, two cultivars were chosen My 55-14 (resistant) and Ba 42-231 (susceptible). Distribution of free and conjugated forms can be related to resistance phenomenon. For studying leaf scald, L55-5 and C439-52 cultivars. were selected. This second cultivar resulted more susceptible on the basis of its phenolic conjugation

pattern; a decrease of free forms in parallel to the increase of conjugated ones.

Yellow leaf syndrome was studied in C120-78 and C87-51 cultivars, both susceptible to phytoplasma. Both cultivars showed a begin of defence response in inoculated plants on the basis of the undergo increase of, principally free, and secondarily conjugated phenolics

P0579. Influence of plant growth regulator obtained on the basis of biocompost on growth parameters and phytopathogen resistance of winter wheat seedlings

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Influence of aqueous extracts and alkaline hydrolysate of biocompost separately and in combination (1:1 ratio) on seedlings growth parameters was studied. In first case the effect was gained due to phytohormones-stimulators presence. Alkaline hydrolysate effect was generated by the means of humic and fulvic acids. Investigated preparation was created on the basis of those products. It was studied as plant growth regulator under the steady state conditions as well as under plants infection with phytopathogens. Seedlings treatments with this preparation partially or fully decrease negative effect of seedling's diseases on growth parameters under the artificial infection. It was established that preparation increases plant resistance to eyes pot, due to papilla formation and decrease fungi penetrations number.

Treatment with the experimental preparation ensures possibility of plant's self-defense potential including mechanism of infection avoidance by the means of papilla formation, fungicidal and fungitoxic effects as well as reduction of pathogens influence on plant growth and productivity due to the preparation's growth stimulatory effect.

P0580. The foliar and cambial responses of *Syzygium cumini* (L.) Skeels to coal-smoke pollution

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This study reveals responses of blackberry tree to varying degree of coal-smoke pollution with specific reference to leaf behaviour and the cambial growth patterns. Leaf area, stomatal size, and stomatal index were markedly reduced under the pollution stress, compared to the control. The chlorophyll and carotenoid contents showed a reverse correlation with the degree of pollution as did the net photosynthetic rate, NR activity, protein content and nitrogen content of leaf. Stomatal conductance dropped to its lowest, whereas intercellular CO₂ increased at the most polluted site. Sugar content declined, showing a negative correlation with sulphur content. The total span of cambial activity did not alter, though the cambial reactivation was delayed by about two months under heavy pollution stress. Duration of phloem formation was reduced, whereas that of xylem formation extended. Annual wood accumulation increased at the polluted site, and the vessel elements, narrower but longer unlike the fibres, occupied a relatively small transectional area in the wood. These changes influenced the vulnerability factor and mesomorphic ratio of the test trees.

P0581. Seed formation in species with underdeveloped embryo *Pulsatilla vulgaris* Mill. and *Helleborus niger* L. (Ranunculaceae)

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Seed formation in *Pulsatilla vulgaris* Mill. and *Helleborus niger* L. characterized by the phenomenon of underdeveloped embryo, was studied. The following common features in this process were revealed: occurrence of endosperm cavity around embryo, zonality in endosperm structure, early accumulation of nutrients in embryo and endosperm, early specialization of seed coat, prolong functioning of micropylar and chalazal seed structures etc. These peculiarities were already noted for other plants with underdeveloped embryos.

The main differences between the species given concern the degree of embryo differentiation at the moment of dissemination (*P.vulgaris* - torpeda-shaped, *H.niger* - heart-shaped stages), the term of certain structures function (antipodes, podium, nucellar cap) and proceeding of some processes (nutrients accumulation, specialization of seed coat elements). This appears to be

associated with different types of seed dormancy that reflects adaptation to various environmental conditions: *P.vulgaris* - forest-steppe plant with morphological seed dormancy; *H.niger* - alpine one with morphophysiological seed dormancy.

P0582. Leaf behaviour, cambial activity and wood production in *Azadirachta indica* A. Juss. as affected by coal-smoke pollution

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The study elucidates responses of neem tree (*Azadirachta indica* A. Juss.) to varying degree of coal-smoke pollution with specific reference to foliar traits and the cambial growth patterns in the stem, as observed in different seasons of an year. Leaf pigment concentrations, NR activity, reducing sugar and total N-content declined, whereas stomatal index and the nitrate and S-contents increased with growing pollution load. Stomatal conductance was low but internal CO₂ concentration of leaf was high, leading to a drop in the net photosynthetic rate under pollution stress. Cambial reactivation in the stem was delayed. Although the total span of radial growth got reduced, more wood was produced at the polluted site. Vessel proportion in the wood increased, whereas size of vessel elements and xylem fibers decreased. Vulnerability factor and mesomorphic ratio were inversely proportional to pollution stress, indicating a situation of growing water stress and a consequent tendency of plants for shifting towards xeromorphy. The mode of carbon-partitioning rather than the photosynthetic rate seemed to have influenced the process of wood formation in the species.

P0583. Effect of chlormequat (CCC) on growth and chemical composition of *Ficus benjamina* L. plant

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In greenhouse trials, *Ficus benjamina* L. plants were subjected to one of the following treatments. CCC at 0, 1000, 1500, 2000 ppm. CCC treatments were added to the growing medium (Sphagnum peatmoss + sand 1:1 v/v) drench, 25 ml / plant bimonthly alternately. CCC at 1000 ppm increased No. of internodes and their length, plant height, No. of leaves and branches, leaf measurements, total leaf area / plant, plant canopy, root length and FW&DW of leaves, branches, shoots, roots and whole plants. Oppositely, it decreased stem diameter, dry leaves/ branches ratio and K% in roots. CCC at 1500 ppm caused an increment in internodes length, carotenoids and phenols contents in leaves, N% in branches& roots and P&K% in branches. Whereas, it decreased stem diameter and T.C. % in roots. CCC at 2000 ppm increased dry leaves/ branches ratio, T.C.% in branches, N&P% in branches& roots and K %, indoles, phenols& amino acids in leaves. While, it decreased stem diameter and DW of leaves, branches, shoots, roots and whole plant.

P0584. Effect of salinity and drought on proteins and antioxidant enzymes in seedlings of two *Astragalus* species

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The genus *Astragalus* is generally considered the largest genus of vascular plants with an estimated 2500-3000 species. The greatest numbers of species are found in the arid, continental regions of Western North America and Central Asia. In this research we investigated the effects of NaCl (0-200 mM) and drought (PEG 4000, PEG 6000 & Mannitol, 0-20%) on the germination percentage and some biochemical parameters in seedlings of *A. squarrosus* and *A. microcephalus*. According to the obtained results seedlings of both species tolerated NaCl up to 150 mM, PEG 4000 and PEG 6000 up to 15 % and mannitol up to 10 %. Protein and proline content was determined by the spectrophotometric method and SDS-PAGE system was used for the study of qualitative changes of proteins under stress and for comparison of protein profile in seeds of different species. Isoforms of some antioxidant enzymes such as peroxidase, catalase and polyphenol oxidase in seedlings were characterized by PAGE.

P0585. Rapid response reactions of plant shoots on changes in sodium chloride concentration at the root zoneN. V. Budagovskaya¹, V. I. Guliaev²;¹Institute of Plant Physiology, Moscow, Russian Federation, ²All-Russian Institute of Genetics and Breeding of Fruit Plants, Michurinsk, Russian Federation.

Use of a highly sensitive method (accuracy 0.07 µm) - laser interference auxanometry - made it possible to study rapid (min) and slower (h) response reactions of leaves and stems of wheat, oat, barley, rice and buckwheat plants on increase and decrease in NaCl concentration at the root zone. Addition of NaCl in increased concentration to the root zone of plants caused a two phase response reaction of leaves: decrease and the following increase in their growth rate in each phase. Duration of the 1st phase was 3-fold shorter than of the 2nd. Growth rate of leaves was restored by the end of the 2nd phase (few hours after addition of NaCl). The 1st phase may be related to rapid adaptive reactions and changes in leaf turgor, the 2nd - to slower adaptive processes - *de novo* synthesis of protectors. Introduction of NaCl in high concentration caused stoppage in leaf and stem growth and shrinking of their tissues (for 30-60 min) as result of dehydration. Washing the roots of NaCl rapidly restored the turgor of leaves and increased their growth rate. The data obtained provide information on dynamics of response reactions of shoots on increase and decrease of NaCl concentration at the root zone.

P0586. New plant type architecture for drought and salt tolerance of rice (*Oryza sativa* L. ssp. *indica*)C. Kirdmanee¹, S. Cha-um¹, P. Supalerkbandit², P. Wanichananan¹, D. Boozaya-angoon³;¹National science and Technology Development Agency, National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand, ²Thammasat university, Pathumthani, Thailand, ³Rice research Center, Pathumthani, Thailand.

Phenotype expressions of aroma rice were evaluated the specific characterizations of drought tolerant, drought sensitive, salt tolerant and salt sensitive lines under *in vitro* environmental control system. Vigorous root system was higher in drought and salt tolerant lines than those in drought and salt sensitive lines. The root length and root area of drought tolerant line were longer than those of drought sensitive line. Number of root and root area of salt-tolerant line were bigger than those of salt sensitive line. The osmotic potential of root increased with increasing the root surface area. High osmoprotectants, proline and betain contents, were observed in the salt-tolerant line, resulting in high osmotic potential of root and high survival under salt stress. Rice composed of vigorous root system; high osmoprotectant and high net photosynthetic rate show the high growth rate and high yield. This new plant type has been used for rice breeding program.

II.

P0587. Physiological aspect of hyacinth response to ethephon

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Effect of ethephon (2-CEPA) on plant growth, physiological properties and floral stalk topple of hyacinth in the course of greenhouse forcing was studied. The significant inhibition of growth and the changes in biometric and anatomical parameters of two cultivars (Carnegie and Jan Bos) were revealed. As a consequence, the floral stalk topple was prevented. This morphogenetic effect was shown to be cultivar-specific, the response of Carnegie was more pronounced as compared with that of Jan Bos. The temporary decrease in chlorophyll content in the leaves of cv. Carnegie was detected. However chl. a/chl. b and chl/s/cars ratios were similar for two cultivars. The prevention of floral stalk topple due to ethylene effect was accompanied by decrease in chlorogenic acid content and increase in carbohydrates and bound forms of cytokinins and abscisic acid in the tissues. This work was supported by the Ministry of Education and Sciences of RF (project HScSc-1864.2003.4) and by RAS (Programme "Fundamental basis of bioresources management").

P0588. A study of the different substratum influence to the root development of *Picea abies* L.Karst. and *Pinus silvestris* L. seedlingsV. Golubovic-Curguz¹, D. Vilotic², M. Veselinovic¹;¹Institute of forestry, Belgrade, Serbia and Montenegro, ²Faculty of forestry, Belgrade, Serbia and Montenegro.

For a successful afforestation it is necessary to be used the nursery plants which are with except well developed over ground parts, and also must have quite well developed root system. The well branched root swelling need the lateral roots of first, second and third orders. Significant for operating all of root system are rootlets responsible for water absorption and minerals supply from the soil.

In the study is shown how strong is the influence of the used substratum type to

development of root system of seedlings in the nursery plantation. The carried out sowing of seed *Picea abies* L. Karst. and *Pinus silvestris* L. in the substratums of peat and humus taken from the natural sources, which were different in the texture and chemical composition.

The experiment applied in the five variations of substratums, pure or in mixed combinations of them. During the fertilization the regular steps of nursering and followed the development of seedlings in all of variants. A morphometric seedling root analysis was done at the end of vegetation.

The results of this study confirmed that the used type of substratum had a very significant influence to the seedling root development.

P0589. Accumulation and intracellular localization of heavy metals in tolerant plants.M. Nadubinska^{1,2}, V. Banasova¹, O. Horak², M. Ciamporova¹, D. Gruber², I. K. Lichtscheidl²;¹Institute of Botany, Bratislava, Slovakia, ²Institution of Cell Imaging and Ultrastructure Research, Vienna, Austria.

Phytocoenological investigations of former mining and industrial sites in Slovakia and Austria revealed species-poor vegetation with a dominance of some heavy metal tolerant plants. Extreme concentrations of Pb, Zn and Cd in soil were found in Arnoldstein (Austria) and Banská Štiavnica (Slovakia); Cu was abundant in Banská Bystrica (Slovakia).

The total and water soluble metal content of plants and soil was measured by plasma emission or atomic absorption spectroscopy. Heavy metal uptake and accumulation differed substantially between accumulator plants (*Thlaspi caerulescens*, *Arabidopsis halleri*) containing more than 1 % of Zn, and excluders (*Silene vulgaris*).

For plant specific localization, we combined tissue-specific EDX - scanning electron microscopy with fluorescent labeling in live cell. A proper fluorescent marker was selected and its concentration assessed. This new approach allowed to localize zinc to vacuoles of leaf epidermis and also to some mesophyll cells of *T. caerulescens*. This correlates well with the finding that more than 70 % of Zn in plant are water soluble.

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P0590. Comparison of leaf anatomical characters in some CAM plants of the Crassulaceae

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The aim of this work was to compare the stomata frequency, the presence/absence and quality of epidermal appendages and the dimensions of the mesophyll cells in five species adapted to different environmental conditions. (*Kalanchoe daigremontiana*, *K. fedtschenkoi*, *K. tomentosa*, *Crassula multicava* and *Cotyledon ladysmithiensis*). These plants have leaves with different level of succulence (ratio of leaf area and leaf thickness). The highest succulence level has been detected in *Cotyledon* plants together with the lowermost stomata number and largest mesophyll cells. The succulence and stomata number of the three *Kalanchoe* species are different. The less succulent *Kalanchoe daigremontiana* bears twice as much stomata, than the former plant. Although the succulence of *K. tomentosa* is near to *Cotyledon*, the stomata number is the highest among species, indicating that there is no direct relation between the stomata number and the succulence. We also estimated the contribution of

the epidermal appendages in the environmental adaptation. The level of the taxonomic relationship is not reflected in the anatomical characters.

P0591. Optimal photosynthetic capacity of nitrogen fixing plants: Is it larger than photosynthetic capacity of nitrogen absorbing plants?

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Some higher plants acquire nitrogen (N) needed for the growth by symbiotic associations with microorganisms that fix atmospheric dinitrogen (N₂). The symbiotic N₂ fixation has been considered to be especially important for plants inhabited in the environments where N severely limits plant growth. However, as do the systems that absorb nitrogen from soil solutions, symbiotic N₂ fixing systems require a great carbon input for constructing and maintaining the specific apparatuses and assimilating N₂. Thus, too much carbon allocation to N₂ fixation decreases plant growth rates, while it could increase N concentration in plants and hence photosynthetic capacity (P_{max}). We presented a model analysis of optimal photosynthate allocation to N fixation and optimal photosynthetic capacity that maximize the growth rate of N₂ fixing plants. The model predicts that optimal photosynthetic capacity of symbiotic N₂ fixing plants is smaller than that of N absorbing plants under a wide range of nitrogen availability. However, this result contrasts to the general observation, which reason is also discussed.

P0592. Morphological and anatomical adaptations of some species of aquatic macrophytes to sazonality of Pantanal Mato Grossense, Brazil.

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Pantanal Mato-Grossense, the biggest flooded plain in South America, is located in middle west of Brazil. In this ecosystem there is a great diversity of macrophyte species belonging to different botanical families. These plants present peculiar morphological and anatomical adaptations, large phenotypic plasticity and, most of them, are amphibian species, occurring even in aquatic environment as in soil free of flood. Species of Onagraceae, Alismataceae, Pontederiaceae and Polygonaceae were collected and prepared to Light Microscopy and Scanning Electron Microscopy. Avoiding species to stress imposed by aquatic environment present frequently typical aerenchyma, heterophylly, submerge thin leaves, bright cuticle, characteristics observed in several species of these families. Some species of Onagraceae and Pontederiaceae are tolerating to stress imposed by aquatic environment. Quantitative and qualitative analysis of morphological and anatomical variations related to flood and dry seasons in Pantanal, and the peculiar aspects of some species are presented.

P0593. Study of quantitative and qualitative changes of proteins and polyphenol oxidase in several plucking from Iranian tea leaves

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Polyphenol oxidases (PPO) can hydroxylate monophenols to o-diphenols and oxidate o-diphenols to o-quinones. The oxidative and hydrolytic enzymes endogenous to tea shoots are crucial for generation of various characteristic quality attributes of black tea. Plant materials of Iranian fresh tea leaves plucked from a farm located in the north of Iran. Protein was determined by the method of Lowry et al. (1951), and Bradford (1976). PPO activity was determined at 410 nm. Electrophoresis, pH optima, thermal stability, Substrate specificity, effect of inhibitors and optimum temperature also studied. We found optimum temperature for sample 1 to 3, 35,40 and 30 °C respectively. Optimum pH was 8.6 for sample 1 and two pH for either sample 2 and 3, 5.2 and 7.2 for 2 where as, 4.8 and 7.9 for 3. In thermal stability sample 2 showed higher thermal sensibility than the others. We found some substrates with no activity with sample 2 but with a high activity in the others. The effect of five inhibitors, namely NaCl, glycine, ascorbic acid, L-cysteine and sodium azide on crude Iranian tea PPO activity were studied. We found all by inhibitory effect with different inhibitory potential.

P0594. Stress-induced flowering in *Arabidopsis thaliana*? Effects of mineral nutrition deficiency on *A. thaliana* flowering.

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Flowering is regulated by many cues (e.g. photoperiod). In some species, it is accelerated by abiotic stresses. This may enable plants to "escape" severe stress by producing seeds. We studied how nutritional stress affects flowering of *Arabidopsis thaliana*.

Ecotypes Col and Ler were supplied with 1% or 100% Hoagland solution (H). In one experiment, plants were continuously grown in these solutions in 2 daylengths (8 h, 12 h). When compared to 100% H, Ler grown in 1% H had fewer leaves at anthesis but flower bud appearance and anthesis were delayed. All these parameters were strongly affected by photoperiod in 100% H but much less in 1% H. Similar results were found in Col.

We also tested the response of Col to a change in nutrient supply. Plants were initially grown in 100% H and the solution was then changed to 1% H. These plants developed flower buds and opened flowers earlier and after making fewer leaves than controls kept in 100% H.

Continuously poor mineral nutrition appears to delay *Arabidopsis* flowering and reduce its sensitivity to photoperiod. In contrast, flowering is accelerated by a sudden decrease of nutrient levels.

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P0595. Structure and ultrastructure of stigmatic papillae of *Datura stramonium* L. during its development

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Structure and ultrastructure of papillae at the surface of stigma of *Datura stramonium* L. (Solanaceae) were investigated using light and transmission electron microscopy. In the course of development, papillae progressively covered the stigma as unicellular structures, then lysed and detached from its surface. Papillae as well as underlying stigmatic cells showed high secretory activity. Secretion began very early in the 20-25 mm long buds. In this stage, electron dense droplets were observed at the surface of the endoplasmic reticulum, in the cytoplasm and in the vacuoles. Electron density of these components after post-fixation with OsO₄ and their capacity for staining with Sudan Black B and Auramine O revealed their lipid nature. In the mature stigma droplets increased in number and formed large lipid bodies under the cuticle and on the stigma surface. This procedure caused the cuticle to rupture and become distanced from the cell wall. It seemed that a part of lipid exudates passed through the ruptures and reached the stigma surface. The existence of these ruptures may facilitate the passage of the lipid material that occurs also through the thin cuticle.

P0596. Sterile mutant of *Verbena hybrida* induced by heavy-ion beam irradiation and wild species *V. peruviana* exhibit self-incompatible phenotype.

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Garden verbenas (*Verbena hybrida*) belonging to the Verbenaceae family are originated from interspecific hybridization among several species, many cultivars frequently produce seeds. Recently, a sterile mutant has been isolated in the verbenas cultivar 'Coral Pink' of Temari series (Suntory Flowers Ltd., Tokyo, Japan) by mutation induction using heavy-ion beams at RIKEN Accelerator Research Facility (RARF, Saitama, Japan). We investigated pollen and ovule fertility assessed by acetocarmine staining, seed-set following controlled-pollination tests and behavior of pollen tubes in pistils with the sterile mutant of 'Coral Pink' (SC) and its original fertile one (FC). As the results, although SC has functional male and female gametes, few self-pollinated flowers carry out seed-set, leading to sterile phenotype. Additionally, the sterile mechanism of SC was compared with the wild species *V. peruviana* (VP) which is one of origin of Temari series and exhibits sterility. Interestingly, similar phenotype was observed in PV. We further investigated, therefore, whether there are differences on self-incompatible reaction between SC and VP.

P0597. An investigation on pollen tube growth and embryo development of tomato (*Lycopersicon esculentum* Mill.)

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In this study the development of the pollen tubes into the styles was investigated using the fluorescence method according to Kho and Baer (1968). For this study, flower buds were emasculated before anther dehiscence and pollinated by hand on the day of anthesis. Ten tagged flowers were collected 3, 6, 12, 24, 48, 72 h after pollination and scored pollen tube growth according to a method by Henny (1981).

For embryo development, flower specimens were taken at daily intervals starting one day after pollination up to ten days. Flowers were fixed in FAA for 24 hours, dehydrated in a graded ethanol series and embedded in paraffin. Sections of ovary in 5-8 µm thickness were stained with Haematoxylen according to Brooks (1950).

After germination on the stigma, pollen tubes developed into callose plugs inside the styles and kept moving in bundles until they reached the carpels.

During the ovule-related experiment, pseudoembryo formation was observed beside normal embryo development.

Detailed results and illustrations will be presented in the text.

P0598. Sperm-Biased Mutation Transmission in Plants: Discovery and Explanatory Models

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Here we report results of two separate studies showing that sperm carry more mutations than eggs in both gymnosperms and angiosperms, and then we present simple models of optimal allocation to repair of sperm versus egg. In the first study, we compared the substitution rates at neutral sites of maternally and paternally inherited organellar DNA in gymnosperms. Sperm were found to transmit a greater number of mutations in both mitochondrial and chloroplastidial DNA. Second, we used UV-B and UV-C to induce deleterious mutations in *A. thaliana*. Analysis of grand-offspring (F2) showed that a successful sperm on average carried more deleterious mutations than a successful egg. These mutations were induced in somatic tissues and were passed to the gametes. Finally, simple models are constructed to study the effects of several factors on the optimal allocation to repair of sperm versus eggs. When benefit-cost curves differ, optimal allocation to repair is lower for the gamete type having the more-efficient repair. In addition, optimal investment in repair of sperm is lower with increased levels of self-fertilization and with decreased levels of inbreeding depression.

P0599. Neogene Plant Cuticles and Environmental Changes from Yunnan, Southwest China

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Neogene and Paleogene plant fossils in China are mostly impression and seldom compression fossils. Recently, abundant plant fossils were found in the Neogene from Tengchong and Baoshan in Yunnan Province, Southwest China. These fossils were collected from the Nanlin Formation (Miocene) and Mangbang Formation (Pliocene). It is noticeable that a lot of specimens have good cuticles owing to conserving condition and fine lithologic character. Among them, some angiosperm cuticles (*Carpinus subcordata* Nathorst, *Ulmus harutoriensis* Oishi et Huzioka, *Quercus pannosa* Hand-Mazz, *Nothaphoebe cavaleviei* Yang and so on) were anatomized. The stomatal parameter of *Carpinus subcordata*, which indicates that atmospheric CO₂ concentration in Neogene, is higher than that of today. By comparing the cuticular characteristic of *C. subcordata* with that of its NLR, *C. cordata*, we conclude that Neogene climate in western Yunnan is warm and humid, belonging to torrid zone to sub-torrid zone or warm temperate zone. This work was supported by the NSFC (40372012), Key Project of Science and Technology of Educational Ministry of China (104177), NSF of Gansu, China (ZS031-A25-002-Z).

P0600. Seed dispersal in the pre-Mediterranean, relict *Coriaria myrtifolia*: an evolutionary interpretation

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The evolutionary history of the plant-disperser mutualism has been rarely examined. In particular, few studies have explicitly examined seed dispersal systems of plant species known to be relict taxa in their present-day environment. Moreover, seed-dispersal biology in the genus *Coriaria* is virtually unknown. Here, dispersal system of *Coriaria myrtifolia* L., a fleshy-fruited shrub, were explored. It is a typical representative of relict plant taxa of Tertiary origin that make up a great part of the present-day Mediterranean flora.

Current relationships between *C. myrtifolia* and its vertebrate dispersers in NE Spain, for summer 2004, were analysed. The main dispersers were found to be mammals, specially *Martes foina*. Seeds were dispersed in apparently unfavourable microhabitats, rarely more than 2-3 m from mother plant.

A primary adaptative role of its infrutescence to endozoochory versus a possible defensive role of fruit pulp is also discussed. Besides, a relationship between fruit-size and dispersers were examined to genus level, on the basis of that fruit size appears to be more sensitive to dispersers-driven selection than other fruit traits

P0601. The *Glyptostrobus europaeus* whole-plant in the Pliocene fossil forests of Italy

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Foliage, seeds and cones of *Glyptostrobus europaeus* (Taxodiaceae, or Cupressaceae s. l.) are very common within muddy sediments in several Pliocene fossil forests of northern and central Italy. Recent investigations in the Stura di Lanzo site showed that the plant bearing the *Glyptostrobus europaeus* foliage, also produced stumps and logs with the recently defined *Glyptostroboxylon rudolphii* wood-type, which is diagnostic for the genus *Glyptostrobus*. Also in the spectacular fossil forest of Dunarobba (central Italy) conifer foliage, seeds and cones belong uniquely to *Glyptostrobus europaeus*. The wood-type, formerly identified as *Taxodioxylon gypsaceum*, has been re-assigned to *Glyptostroboxylon rudolphii*. A third analogous example concerns a fossil forest preserved in the Pliocene succession of the Upper Valdarno basin. The numerous and huge upright trunks of these three sites provide a good example of what was the habit of the *Glyptostrobus europaeus* whole-plant, the more common and largest tree growing in the local swamps at that time.

P0602. Yakima Canyon permineralized plants: a lens into the Miocene

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The Yakima Canyon of central Washington state, USA contains a diverse middle Miocene permineralized "bog" flora of fruits, seeds, leaves and other remains. The suite of taxa occurring at the different florules within the flora represent a wetland (swamp) community and elements from the adjacent slopes. Our studies of the past decade have provided highly detailed systematic information about several genera at Yakima, including *Woodwardia*, *Liquidambar*, *Quercus* and *Taxodium*. Also present are *Vitis*, *Nyssa*, *Paliurus*, *Pinus*, *Osmunda*, *Meliaceae* and the newly described lythraceous fruit *Shirleya*. We now wish to use the plants from the Yakima flora as a focal point for interpreting the diversity of genera that are also known as compressions, pollen and petrified wood in the Columbia River Basin. From this study we will gain a better understanding of the Neogene fossil record of several important taxa and their critical taxonomic characters.

P0603. Diversity and dynamics of Holocene mangroves of the western Ganga Brahmaputra Delta, India.

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The coastal areas of western GB Delta are covered by extensive mangrove forest, the Sundarbans. The forest is considered as the world's largest single continuous tract of mangrove forest. *Avicennia* is the dominant forest type followed by *Excoecaria*, *Phoenix* and *Ceriops*.

Pollen analytical studies on sediment deposits collected from different parts of the western GB delta also reveals the presence of a mangrove ecosystem in the area since the last 9000 years. Palynological data indicates rapid sea level change during the

early to mid Holocene limited mangrove ecosystems on the southernmost plain of the GB Delta. From the mid-Holocene onward, mangrove forests spread south eastward. The development of mangrove forest during this time has been in response to altered sedimentation rates as sea level rise decelerated and increased rate of sediment accumulation came into balance with the rate of subsidence. As a result of these factors, along with high fresh water influx after the mid Holocene, progressively more luxuriant growth of mangrove began to inhabit the southern and south eastern Sundarban region to along what is now the present day coast.

P0604. History of formation of forests in the plain part of Ukraine in the Holocene

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The authors analyzed and generalized the results of palynological and radiocarbon-dating studies of Holocene deposits of the forest, forest-steppe and steppe zones of Ukraine. Based on the obtained data, we reconstructed the pattern of main changes of vegetation and climate starting from 10,300 years BP. We consider changes in forest vegetation of the studied area in the Early (PB-1, PB-2, BO-1, BO-2, BO-3), Middle (AT-1, AT-2, AT-3, SB-1, SB-2, SB-3) and Late Holocene. For most important forest-forming trees (species of *Pinus*, *Betula*, *Alnus*, *Quercus*, *Tilia*, *Carpinus*, *Fagus* etc.), the main periods of their maximum participation in Ukrainian forest vegetation were identified. Broadleaf forests and mixed forests with participation of broadleaf trees were most widespread in Ukraine 4,500-6,200 years BP. During the second phase of the Atlantic time of the Holocene the northern border of the steppe zone in Ukraine was stable. Expansion of forest communities in the steppe zone progressed through gradual increase of forest areas that originally occurred in floodplains and ravines.

P0605. The Late Pleistocene/Holocene record of environmental history from the Bulgarian Black Sea coast

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Lacustrine sediments of eight coastal lakes and marine sediments of 17 cores from the shelf, continental slope and deep-water area were subjected to pollen and dinoflagellate analysis and provide Late Pleistocene/Holocene record of vegetation dynamics, human impact, and climatic changes along the Bulgarian Black Sea coast. According to pollen record open steppe communities dominated by cold resistant and heliophilous taxa such as *Artemisia*, *Chenopodiaceae* and *Poaceae* prevailed during the Pleniglacial and during the Stadials of the Late Glacial. Climate must have been cold and dry. Only during the Bølling/Allerød Interstadials of the Late Glacial the increase of *Pinus diploxylon* indicates certain improvement of climate. Two lines of vegetation dynamics during the Holocene could be distinguished. Along the Northern coast xerophytic communities dominated. Only during the climatic optimum (Atlantic) steppe-forest communities occurred. Along the Southern coast the migration of arboreal taxa started after 9945±160 BP from their Late Glacial refugia. Human impact from the Eneolithic and Early Bronze Age is also registered.

P0606. Evolution of plastid genomes within the parasitic flowering plant genus *Cuscuta*

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The holoparasitic plant genus *Cuscuta* comprises species with full photosynthetic capacity, and functional chloroplasts, achlorophyllous species and intermediate forms with restricted photosynthetic activity and degenerated chloroplasts. Compared to nonparasitic plants, extensive deletions have taken place in the plastid DNA of achlorophyllous species encompassing photosynthetic as well as regulative genes, while green species like *C. reflexa* have retained most of the plastid genome. Transcription of the *C. reflexa* ptDNA resembles that of nonparasitic higher plants regarding promoter structure and transcription patterns but shows marked differences in the steady state RNA profiles. In intermediate species, genes for the photosynthetic apparatus were retained while the *rpo* genes coding for the plastid-encoded RNA polymerase that is responsible

for their transcription were lost. Concomitantly, adaptations within the plastid genome have occurred that enable transcription mediated exclusively by a nuclear-encoded plastid RNA polymerase. Thus, *Cuscuta* is an interesting model for the evolution of reduced organellar genomes.

P0607. Phylogenetic relationships among algal streptophyte and embryophyte lineages based on chloroplast genome evolution

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Our understanding of chloroplast genomic evolution has expanded by the increased availability of complete genomic sequences in green plants. The known embryophyte introns and five operons in about 30 genera from all major lineages of algal streptophytes were surveyed and analyzed. The survey indicates that Streptophyta sensu lato cpDNAs contain *psbB* and *rpl23* operons, and that derived charophytes are distinguished from primitive charophytes by the presence of introns previously only found in embryophyte cpDNAs. Derived charophytes have similar operon structures involving *clpP* and 3' *rps12* operons that are not found in primitive charophytes. In addition, the complete sequences of two charophyte chloroplast genomes suggest that the five operons and all embryophyte introns are of a charophyte origin. However, some algal streptophyte lineages experienced gene transfer, intron loss, and/or genomic rearrangement possibly associated with the loss of inverted repeats. Phylogenetic relationships of algal streptophytes and embryophytes were constructed using genomic changes including the gain/loss of *matK* and the loss of chloroplast *rpl5*, *chl1*, and *tufA*.

P0608. The chloroplast genome of the charophycean green alga *Chara vulgaris*

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It is well recognized that land plants arose from green algae belonging to the Charophyceae. Of the five orders recognized in this class, the Charales and Coleochaetales are thought to be the closest relatives of land plants. We compared the chloroplast DNA (cpDNA) sequence of *Chara vulgaris* (Charales) with those of *Chaetosphaeridium* (Coleochaetales) and the liverwort *Marchantia*. Like the latter cpDNAs, *Chara* cpDNA features a quadripartite structure with two copies of a rRNA-containing inverted repeat separating large and small single-copy regions. At 184,933 bp, *Chara* cpDNA is substantially larger than *Chaetosphaeridium* and *Marchantia* cpDNAs. Considering that all three genomes share a very similar gene repertoire, the lowest density of coding sequences is found in *Chara* cpDNA. Gene order is highly conserved in the three genomes. Two group I and 16 group II introns reside in *Chara* cpDNA; 13 of these introns share homologs at identical positions in both *Chaetosphaeridium* and *Marchantia* cpDNAs. Overall, our results support the idea that the typical features of land plant chloroplast genomes were gained in the green algal ancestors of all terrestrial plants.

P0609. Complete structure of the chloroplast genome of the duckweed, *Lemna minor*

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We determined the complete nucleotide sequence of the chloroplast genome of the duckweed, *Lemna minor* (araceae, monocots). It is a circular DNA molecule, 157 kb in size, and is composed of a large single copy region of 17 kb, and a pair of inverted repeats regions of 26 kb each. We compared the sequence to other published chloroplast genomes to infer the location of putative genes. When the inverted repeat is considered only once, we assigned 115 genes, of which 81 could encode proteins, 30 encode tRNAs and 4 encode rRNAs. Most putative protein coding genes appear to start with an ATG codon, but we also detected other possible start codons, some of which, as well as appasent stop codons in the putative genes, suggest RNA editing. A comparative analysis among angiosperms showed that the *Lemna minor* chloroplast genome was more similar to that of some dicots (*Calycanthus floridus*, *Panax schinseng*, *Nymphaea alba*, etc) than to chloroplast genomes of monocots belonging to

the grass family (rice, maize, wheat and sugarcane). This work was supported by a grant from the Russian Academy of Sciences.

P0610. Comparative phylogenetic analyses of cryptophyte nuclear, nucleomorph and plastid genes

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The ancestor to the plastid-bearing Cryptophyceae obtained its plastid by engulfment of a red alga. The reduced former red algal nucleus, the nucleomorph, was retained in the cryptophyte cells between the two outer and inner plastid membranes. Thus, cryptophytes are chimaeras with four genomes in a cell. In this study, genes of the nuclear and nucleomorph ribosomal operons and the plastid gene *rbcl* of several cryptophyte taxa were sequenced and their evolutionary rates and phylogenies compared. In the DNA sequences of *rbcl*, the trees were best resolved with data sets including third codon positions despite of an obvious bias. Seemingly codon biases may support molecular phylogenies in highly conserved sequences of closely related taxa. Whereas the phylogenies were largely congruent in nuclear, nucleomorph and plastid gene phylogenies, differences were found in evolutionary rates of the genes predominantly in clades containing leukoplast-bearing taxa. Possibly nucleomorph and plastid genes of the cryptophytes coevolve as a unit under similar evolutionary constraints.

P0611. Palynological characteristic of the Middle and the Upper Jurassic of the Russian Platform

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The Bathonian, Callovian, Oxfordian palynological assemblages from central region of the Russian Platform are described. The Lower Bathonian assemblage is characterized by predominance of spores (69%), mainly Cyatheaceae (30%) and Gleicheniaceae (8%). The pollen of *Classopollis* occurs only in minor quantity (1,2%) in the Lower Bathonian assemblage and up to 4% in the Upper Bathonian one. The various ancient spores (*Neorastickia rotundiformis*, *Toroisporites* sp., *Sterisporites incertus*) separate the Lower Bathonian assemblage from others. Five palynological assemblages are distinguished in Callovian deposits. The amount of *Classopollis* is increased up to 33-51% which reflects in warming of climate. Two abundance peaks of marine dinocysts and acritarchs occur in ammonite zones *Proplanulites koenigi* and *Quenstedtoceras lamberti* that indicate two transgressive events during the Early and the Late Callovian Ages. The Oxfordian assemblage is also dominated by dinocysts and green algae (up to 60%). The marine basin had been getting more deep-water for the Oxfordian time.

P0612. Meristem development and dichotomous branching in *Selaginella martensii*

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Dichotomous branching is ancestral in vascular plants, and persists in both lycophytes and euphyllophytes. Shoot branching in *Selaginella martensii* was analyzed over time, using sections and a non-destructive sequential replica technique. *Selaginella martensii* exhibits anisotomous dichotomous branching in which the shoot apical cell (AC) is short-lived, determinate, and not directly involved in the formation of either leaf primordia or new branch shoot apical meristems (SAMs). Early growth of new branch SAMs occurs by the activity of a group of apical initials, rather than a single AC, which forms later. The number of cutting faces of an AC (2, 3 or 4) and the direction of AC segmentation (clockwise or counterclockwise) are unrelated to each other and to these attributes of the AC of preceding or successive dichotomies. AC presence and activity in this species varies with the cycle of dichotomous branching.

P0613. The first finding of a new lycopod cone from the Lower Carboniferous of Moscow Region (Russia)

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A new lycopod cone found in a clay shale of the Serpukhovian deposits of Moscow Region. It is attached to a shoot, on which

short subtriangular leaves are helically disposed. A lower lateral branch of the shoot bears a spherical strobilus, which is very unusual for Carboniferous lycopod cones. Its apex is a rounded and its base - a wedge-shaped. The strobilus reaches a length of 116 mm and a width of 92 mm. Lanceolate sporophylls with acuminate tips are in the upper part of the strobilus. The strobilus is heterosporous. Mega- and microsporangia are not arranged regularly respect to each other. Numerous microspores of *Lycospora*-type are seen in the distal part of ovoid microsporangia. They have rounded outlines and reach 20-25 µm in diameter. More than one tetrad of the megaspores of the genus *Sublagenicula* are present in megasporangia. Each megaspore has a top shaped as a subgula. Several isolate megaspores and their tetrads are seen in different areas of the strobilus. Megaspores from the strobilus largely vary in their sizes. The research was supported by Russian Foundation for Basic Researches No 03-04-49351.

P0614. Mycorrhizae and Land Plant Evolution

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The successful colonization of the land by plants (embryophytes) is an intriguing subject for plant and evolutionary biologists. The mycorrhizal symbiosis between plants and fungi has been suggested to play an important role in this process. We have conducted a literature survey of distribution of mycorrhizae and mutualistic plant-fungus symbiosis in most families of land plants and mapped these data onto a land plant phylogeny reconstructed using molecular data. This analysis allows assessment of phylogenetic depth and frequency of evolution of mutualistic plant-fungus symbiosis and mycorrhizae. We are also investigating evolution of several plant genes, DMI 1, DMI 2, and DMI 3, which are involved in mycorrhizal symbiosis. We aim to determine their time and mode of origin possibly in charophytes and bryophytes, and to examine their patterns of evolution throughout land plants. These data will help to shed light on evolution of genetic basis of this important ecological trait that enabled plants to invade the land.

P0615. *Ashicaulis* (Osmundaceae) from the Middle Jurassic, northern China

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Ashicaulis (Osmundaceae) is described on the basis of one small-silicified rhizome collected from the Middle Jurassic Tiaojishan Formation in Hebei, China. This specimen consists of a small, upright stem surrounded by frond bases, and adventitious roots. In cross section, the stem ranges from 8.5 to 10.5 mm in diameter with a dictyoxylous xylem cylinder composed of 24-32 xylem strands separated by many leaf gaps. The stem consists of parenchymatous, and a two-layered cortex. Stipular wings have one large sclerotic mass. This is the third structurally preserved rhizome of *Ashicaulis* from the Mesozoic in northern China. Two fossil species of the genus *Ashicaulis*, *A. hebeiensis* and *A. liaoningensis*, have been described from China and more than 20 species have been found in the higher latitudes of the southern hemisphere, India, and USA, from the Triassic to Early Cretaceous. New species of *Ashicaulis* adds the further evidence for reconstructing past diversity and geographic distribution of the Osmundaceae, especially in Eastern Asia.

P0616. Origin and evolution of *Ephedra* (Ephedraceae, Gnetales)

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Ephedraceae include a single genus (*Ephedra* L.) and ca. 50 species worldwide. Huang and Price (2003) believe that extant *Ephedra* originated approximately 8-32 Myr ago, but fossil evidence from our study clearly suggests a much older origin. We recently described a new fossil species of *Ephedra* from the Jianshangou Bed in the lower part of the Yixian Formation at Huangbanjigou village, Shangyuan district of Beipiao City, western Liaoning Province, northeast China, which is aged about 126-145 Myr. This new species is closely related to the extant *Ephedra rhytidosperma* Pachomova because they share some reproductive characters, e.g. ovulate cones bearing 2 pairs of bracts and the

unique ovules/seeds surface sculptural characters. This finding coupled with other recent discoveries clearly suggests that *Ephedra* has a much older age than previous estimation from molecular studies, and the family Ephedraceae may be more diverse in the Lower Cretaceous/Upper Jurassic.

P0617. Genetic variation and SNP analysis of modern melons compared to a medieval sample

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aDNA of medieval (15th cent.) melon (*Cucumis melo*) were analyzed and compared to modern melon cultivars (47) with a final attempt to reconstruct the extinct melon. SNPs (single nucleotide polymorphism) were detected in the transcribed spacers (ITS1 and ITS2) of ribosomal DNA (rDNA). Eight of the twenty microsatellite markers amplified a total of 485 SSR alleles. The number of alleles per marker ranged from 2 to 7 with an average of 5.7 including CMCT44 (2 alleles), CMAG59 (5 alleles), CMGA104 (5 alleles), CMCT134 (4 alleles), CMTA134 (6 alleles), CMCTT144 (7 alleles), CMTC168 (6 alleles) and CMCT170 (5 alleles). Sequence analysis of SSRs showed different fragment lengths depending on changes in the number of unit of core sequences. Dendrogram (SPSS11) based on the presence versus absence of SSR and ITS alleles revealed that medieval melon had characters of *inodorus* type melons. Source of grants: OTKA-M-04563, OECD-JA00018642, USA-Hungarian Fulbright Commission, and SzÖ-4/2003.

P0618. Cap analysis of common millet cultivars compared to a 15th century sample

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Common millet (*Panicum miliaceum*) seed remains were excavated from the 15th CENT. layer (King's Palace, Budapest, Hungary). The extracted aDNA was analyzed by ISSR and SSR marker and compared to twenty modern c. millet varieties. SSR sequences at *gln4*, *sh1*, *rps28* and *rps15* loci; and a mitochondrial (mtDNA) fragment (*Mbol*) at 18S-5S-rDNA locus was recovered and sequenced from the medieval millet. Four (*gln4*, *sh1*, *rps28* and *rps15*) SSR markers amplified orthologous alleles in the 15th CENT. millet. After sequencing the SSR alleles showed identical alignment to modern millet with only one SNP at the 6th position (A to G) at *rps28* locus. CAP analysis revealed no any SNPs in the restriction sites of six endonucleases *TaqI*, *BsuRI*, *HinfI*, *Mbol*, *AluI* and *RsaI*. After cluster analysis, middle age millet showed the closest genetic similarity to a Russian registered modern variety 'Omskoje-9'. Source of grants: OTKA-M-04563, OECD-JA00018642, USA-Hungarian Fulbright Commission, and SzÖ-4/2003.

P0619. The Preservation of Cytoplasm in Fossil Plants

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Cytoplasm is a rare object for paleobiological research. The cytoplasm in extant plants usually is decayed shortly after death. However, this decay process needs to be re-examined in light of new data. The decay of cytoplasm is an organic reaction that needs time and the action of enzymes that are very sensitive to temperature changes. Wildfires are not rare at all geological history. High temperatures of forest fires can stop the decay of cytoplasm and the right combination of temperature and occurrence of the fire can charcoalify plant bodies, including cytoplasm. Charcoal is an inert material frequently seen in the sediments. Based on these knowledge, it is conceivable that cytoplasm fossils should be more common objects for research than previously thought. The never-seen-before fine preservation and ultrastructures of cytoplasm in charcoalified plant debris from the early Cretaceous in Kansas, USA are presented in this paper. Different preservation patterns suggest that at least lightning and wildfires are related to the production of fossil cytoplasm. This conclusion is favored by the experiments with modern plant materials.

P0620. Mitochondrial DNA of early land plants: introns, exons, and spacer evolution in bryophytes

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Bryophytes as the basalmost embryophytes play a crucial role in the understanding of mitochondrial evolution in land plants. Intron conservation in the mitochondrial genes *nad2*, *nad4*, *nad5* and *nad7* corroborates the distinctness of the bryophyte classes mosses, liverworts, and hornworts. Positionally conserved introns then provide variable nucleotide sequences which are a useful tool for phylogenetic analyses on lower taxonomic ranks. Few is known so far about conserved gene arrangements in bryophyte mitochondrial DNA.

The gene cluster *nad5-nad4-nad2* is known to be co-transcribed in *Marchantia polymorpha* (liverwort) and conserved in the alga *Chara*. We have investigated this gene continuity in all three bryophyte classes and observe striking differences in the modes of evolution of the two closely located gene spacers flanking *nad4*. Analyses of this and other gene arrangements such as the *trnA-trnT-nad7* cluster give further insights into evolutionary processes in early land plants.

P0621. Micromorphology of trichomes of *Thymus bracteosus* (Lamiaceae)

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Thymus bracteosus Vis. ex Bentham (Lamiaceae) is an endemic species distributed at Dinarides. Morphology and distribution of trichomes on the vegetative and reproductive plant parts were investigated using light microscopy and scanning electron microscopy. The plant material was collected during summer 2004. The leaves of *T. bracteosus* bore an indumentum of unicellular papillae, unbranched nonglandular trichomes and glandular peltate trichomes on different stages of development. Numerous juvenile and rare mature peltate trichomes were distributed in epidermal depressions on both leaf sides and on the outer calyx surface. Nonglandular, unbranched, elongated trichomes are especially densely distributed on stem and calyx, while on the leaves were distributed only on leaf margins. Unicellular papillae are distributed on both leaf sides and on the outer calyx surface. The characteristic feature for this species were numerous unicellular papillae which were present on both leaf sides. Distribution and taxonomic significance of various trichomes on vegetative and reproductive parts of this and related, previously investigated species, is discussed.

P0622. Epicuticular secretions on seed surfaces of Massonieae (Hyacinthaceae)

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The Massonieae Baker is a medium sized tribe of Hyacinthaceae subfam. Hyacinthoideae comprising about 16 genera and 230 species distributed from Africa south of the Sahara to Madagascar and India. In the course of our SEM studies we found different types of epicuticular wax cristalloids on the seed coat surfaces in 6 of 14 investigated genera. Wax cristalloids are a common feature of shoot and leaf surfaces of angiosperms. But on seed coat surfaces they only have been reported in Orchidaceae and in five families of non monocotyledonous lineages. Analysis of non-coding and coding plastid sequences and of intron structure yielded a monophyletic group only when the genus *Pseudoprospero* was excluded from Massonieae. The remaining taxa can be grouped into 9 highly supported clades (bootstrap support value >90%) and thus present a robust framework for mapping of morphological characters onto a phylogenetic tree. The combination of micromorphological character states with molecular data allowed us to discuss the evolution of such traits in a phylogenetic framework and could be a further step towards a better understanding of Massonieae.

P0623. Morphological variability of leaves in some artificial Birch hybrids.

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The aim of the study was to compare the shape of leaves in artificial hybrids. We studied hybrids from silver birch (*Betula pendula* Roth, diploid $2n = 28$) and downy birch (*B. pubescens* Ehrh., tetraploid $2n = 56$) crossing. We examined this progeny on the basis a 22 - year - old field trial. Mother trees of this two species were chosen in Voronezh State Nature Reserve. The choice was random. Seed progeny of the 1st and 2nd generation received by hybridization, self- and spontaneous pollination are grown in experimental stands. The leaves of hybrids were further examined using morphological method. Ten the leaf characters and pollen size were observed. The leaf characters were scored from 15 randomly collected leaves of one hybrid. Some of the hybrids had *B. pubescens* morphology as it was expected and some - *B. pendula* leaf morphology. Only one tree showed continuous morphological variation connecting parent plants. Endogenetic variability of the leaves is not connected with variability of pollen size in this tree.

P0624. Transport-dependent evolution of leaf structure in dicots

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Comparative studies of minor veins and their adjacent tissues in leaves of dicotyledonous plants have resulted in the development of a structural and functional typology and a database on phloem structure in dicots. Two main lineages of evolution of leaf structure correspond to woody and herbaceous dicot species, respectively. The structural characteristics of the phloem are specific for families, sometimes even for orders. With respect to minor vein phloem, an ancestral type (combining traits of both symplastic and apoplastic loading mechanisms), and four specialized types - symplastic (primitive and advanced) and apoplastic (primitive and advanced) - were described. The combination of symplastic and apoplastic features is typical for the Prodicots. Specialized types appear in phylogenetically advanced taxa of the Eudicots: symplastic types in species inhabiting forest ecosystems, and apoplastic types in inhabitants of herbaceous ecosystems. An influence of compartmentation of water flux on the organization of leaf tissues has been demonstrated. The basis for the transport-dependent characteristics of leaf structure has been elucidated.

P0625. Characterization of a blueberry hybrid progeny (*Vaccinium corymbosum* L. x *V. ashei* Reade) in SW Spain by using molecular (ISSR) and morphological traits: congruence between both evidences.

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A first step in a blueberry breeding program currently developed in SW Spain, has been the characterization of the initial germplasm, 38 blueberry hybrid plants produced in EEUU by hybridization between commercial highbush (*Vaccinium corymbosum* L.) and rabbiteye (*V. ashei* Reade) cultivars, with "Misty", "O'Neal" and "Sharpblue" cultivars as control. Morphological and molecular evidences have been examined. In morphological characterization 30 quantitative and 13 qualitative traits have been considered. Multivariate ordination analyses (Principal Component and Correspondence Analyses) and classification (Cluster Analysis) have been performed, and a dendrogram was obtained from the distance matrix. Molecular characterization has been investigated by using the Inter Simple Sequence Repeat (ISSR) technique. The 10 most informative primers of UBC set 9 were selected and generated 151 ISSR polymorphic bands; a dendrogram was obtained based on the similarity matrix. Classification schemes generated by morphologic ISSR data are not coincident. Nevertheless, certain congruence was found in the identification of the most distant groups.

P0626. Development of novel low copy nuclear markers for phylogenetic reconstructions at both genus and species level

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At the species level, the resolving power of both traditional sequential markers (e.g. *rbcL*) and anonymous multilocus markers (e.g. AFLP) frequently becomes limited. Especially when using a low number of sequential markers, problems connected to incongruence can significantly decrease the robustness of the phylogenetic reconstruction.

We developed novel low copy nuclear markers able to allow amplification from a wide array of angiosperm families. Their application to solve phylogenetic relationships is shown for some genera belonging to Lamiales and in particular for species belonging to the *Melampyrum* genus as an example of closely related species within genera. The phylogeny of seven *Melampyrum* species, never determined before, was obtained by means of 8 newly developed nuclear markers in addition to traditional ones, like *ITS*, *matK* and *rbcL*. The possibility to increase robustness of the phylogenetic reconstruction by using a higher number of sequential markers is discussed.

P0627. Towards embryology of Campanulaceae in connection with family systematics

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Campanulaceae is one of the families with unclear systematic position. The typical genus *Campanula* is of special interest; it is heterogeneous, distributes in almost all Holarctic zones and also in mountain regions where the evolution of *Campanulaceae* mainly occurred. Embryology of different genera members was researched on light level. Taking into consideration Linnaeus statement on significance of frutification and seed structure as important features for definition of systematic position of species and genera within the family, we paid attention to the study of seed structure in fruit (scanning microscopy). Ultrastructure of seed surface is closely connected with the structure of seed coat and is a reprint of outer epidermal and inner spermoderm layers. The most heterogeneity in seed surface ultrastructure is observed in the members of *Campanulaceae*. Great differences in shape and seed sizes were noted that could be also used as the important morphological feature in species description. Resemblance and differences in some embryological features and data on seed surface structure contribute much in systematics and phylogeny of *Campanulaceae*.

P0628. Azolla: Phylogenetic History, Divergence Times, and Character Evolution

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The economically important fern genus *Azolla* (Salviniaceae) comprises a small (seven species) but distinct lineage within the morphologically diverse heterosporous water ferns (Salviniaceae + Marsileaceae) - one of the three major clades of core leptosporangiate ferns. The five extant genera of heterosporous ferns have been largely ignored in systematics studies and infrageneric relationships are virtually unknown. Here, we present results from the phylogenetic analysis of a six-locus data set of coding and non-coding regions from the plastid and nuclear genomes of *Azolla*. By integrating critical heterosporous fern microfossils as age constraints, we are able to determine divergence time estimates for extant *Azolla* species and for the *Salvinia-Azolla* split using our molecular data set. Within an explicit phylogenetic context we also investigate morphological character state transitions that may have been associated with the ecological specialization of these ferns to aquatic habitats.

P0629. Micromorphological analysis of *Stachys scardica* trichomes

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Micromorphology and distribution of trichomes on stem, leaves and calyx of *Stachys scardica* (Griseb.) Hayek were investigated using light microscopy and scanning electron microscopy. This species is widely distributed in Balkan peninsula. The investigated material was collected during summer 2004, from Zlatibor mountain. On the stems two types of trichomes were found: nonglandular 3-armed elongated trichomes were densely distributed, and glandular peltate trichomes with 4-celled heads. Abaxial and adaxial leaf surfaces were covered with numerous multi-branched and elongated nonglandular trichomes and

glandular peltate trichomes with 4-celled heads. The outer surface of calyx was densely covered with numerous multi-branched nonglandular trichomes, among which the peltate glandular trichomes were distributed. From inner side very long simple trichomes were observed to emerge. The genus *Stachys* shows wide range of variability within some species. Usefulness of trichome microcharacters taxonomical treatment an related species is discussed.

P0630. Are all phylogenetic problems possible to resolve if long enough sequences are used?

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It is well known that when the internal branch lengths are short relative to the terminal branches, phylogenetic inference is a very difficult problem. Most real examples of this have focused on deep phylogenetic relationships as the major eukaryot groups, plastids of different phyla, etc. Here, we study the plastid phylogeny of a subset of 8 closely related taxa in *Silene* (Caryophyllaceae). An aligned matrix of 24000 chloroplast DNA characters was analyzed. Despite the low overall variability (>90% constant sites) and the long sequences, some of the internal branches cannot be resolved confidently. This indicates saturation among the few variable sites and/or extremely short internal branches. The amount of homoplasy is very high for a chloroplast data set of 8 taxa (CI=0.6). We have partitioned the data into synonymous/non-synonymous sites, coding/non-coding regions, indels/substitutions, and stem/loop regions of self-splicing introns, to investigate this further. We also evaluate the possibility of recombination between plastid lineages.

P0630. On the modes of neotenic divergence in limens of genus *Gagea* (Liliaceae)

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In the genus *Gagea* the laws of age-related variation of radical leaf permits identification with manifestation of the neoteny phenomenon. The neoteny has been the leading evolutionary trend of this phylon. Intrageneric neotenic divergence generated the large genus (about 250 species) with many morphological features of juvenilization, are presented at the definitive stage. The degree of neotenicization are determined by the correspondence with the empirical standard series of neotenicization, which reflects: age-related leaf blade variation during ontogenesis, degree of neotenicization, and sequence of the individual morphogenesis of the basipetally developing leaf. Besides the 10 stages, the phytomer in the end of its neotenicization, creates two more states: a rudiment stage and the category of «silent» phytomers, when its function begins to be carried out by the next below-located axial phytomer. The algorithm of basal neoteny, of the leads historically to the oligomerization, explains of the localization of «silent» phytomers at the infrabasal, and the suprabasal levels, and evolution of polycyclicity in the direction of monocyclicity of ephemers.

P0631. The PBI Solanum project - an international collaboration to monograph Solanum

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Large plant genera typically lack up to date, critical monographic treatments due to the practical difficulties of studying them in their entirety. The most recent comprehensive worldwide monograph of *Solanum* (Solanaceae) was completed over 150 years ago, and accounts for only a small proportion of the estimated 1500 species currently recognised. The PBI Solanum project is one of four major monographic projects funded by the US National Science Foundation and the All Species Foundation and aims to provide a complete species-level taxonomic monograph of the genus. It is a collaboration between the University of Utah, the Natural History Museum, London, the New York Botanical Garden and the University of Wisconsin-Madison, as well as a number of

Solanaceae specialists worldwide. Advances in the dissemination of information via the internet are being harnessed to provide a web-accessible database of species names, plant descriptions and literature, interactive identification keys, digital images and a database of herbarium collections, as well as providing links to Solanaceae resources in the fields of molecular biology and genomics.

P0632. Flow cytometric analysis of nuclear genome size - the problematic effect of cytosolic compounds

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In plant sciences, flow cytometry is mostly focused on ploidy and nuclear DNA content analysis. Although considered the most reliable technique for this type of analyses, recent studies revealed some problems associated with the methodologies used to isolate and stain plant nuclei. These studies alerted to the presence of cytosolic compounds that can bias genome size estimates. It is therefore important to identify these compounds and develop strategies to overcome this problem.

Until the moment the effect of caffeine and tannic acid was tested in nuclei isolated from *P. sativum*, a usual plant DNA standard, using four of the most popular buffers used for preparation of nuclei suspensions. Caffeine decreased propidium iodide fluorescence in the analysed buffers and tannic acid besides that also affected nuclei morphology. The effect of tannic acid in isolated nuclei was very similar to the effect observed when problematic species, as *Quercus suber*, were analysed.

Further studies with other cytosolic compounds and plant species are in preparation in order to clarify this problem and obtain truly reliable procedures to analyse nuclear DNA content of plant cells.

P0633. Genome variation in the plastid DNA of the mycotrophic orchid *Neottia nidus-avis*

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Chloroplast structure and function, as plastid DNA (cpDNA) sequence, have, with few exceptions, remained highly conserved within plant kingdom. Parasitic plants represent a remarkable exception to this general trend, because much of their plastid genome is under relaxed functional constraint. In this context, the presence of different trophic modes in Orchidaceae, from autotrophic to holoparasitic, makes this group ideal for investigating molecular changes in cpDNA that accompany the evolution of heterotrophy. We investigated nucleotide sequence of a cpDNA region of the mycoparasitic *Neottia nidus-avis* and of its sister species *Listera ovata* in order to detect patterns of evolutionary changes in the genome of a non-photosynthetic plant compared to the corresponding closest photosynthetic taxon. In *Neottia* cpDNA we detected many deletions but we also found the contemporary presence of three non-functional copies of the *rbcl* maintained as pseudogenes. These results indicate that cpDNA in non-photosynthetic plants can easily evolve when functional selection is relaxed and that multiple deletions are not the only main mechanism of structural changes.

P0634. Genome size in *Dahlia* (Asteraceae - Heliantheae)

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Dahlia is a monophyletic genus classified into 4 sections, which are not monophyletic according to a recent DNA phylogeny (ITS of 18S-26S rDNA). We studied variation of genome size (DNA C-values) with regard to taxonomy, polyploidy, crossability, and technical difficulties of measurement in flow cytometry and Feulgen densitometry caused by secondary metabolites. DNA 2C-values ranged from 3.3 pg in *D. dissecta* (2n = 34) to ca 8.9 pg in *D. variabilis* cultivars (2n = 64), which had fairly stable C-values. The highest 2Cx-value occurred in *D. imperialis* (5.1 pg, 2n = 32). Tetraploid *D. coccinea* (2n = 64) shows somewhat reduced Cx-values compared to *D. coccinea*, 2n = 32, but resembles its probable derivative *D. variabilis* (2n = 64). Hybrids correspond to expectation from parental C-values (*D. coccinea* x *D. rudis*, 2n = 32; *D. apiculata* x *D. tenuicaulis*, 2n = 32). Secondary compounds

occasionally caused problems with the Feulgen method, but floral pigments were found relatively inert.

P0635. No DNA Loss in Autotetraploids of *Arabidopsis thaliana* and *Aegilops longissima*

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To address the issue of genome evolution in autopolyploids, and particularly to investigate whether rapid sequence elimination also occurs in autopolyploids as in allopolyploids, we employed AFLP fingerprinting to examine small number of genomic loci in F1 hybrids between two different autopolyploids of *Arabidopsis* ecotypes. Using this approach, we found perfect additivity in the F1 hybrids between the newly formed autopolyploids when compared to their parental lines. Using flow cytometry, we extended our study in a quantitative manner, in which the nuclear DNA contents in two newly formed autopolyploids, namely *Arabidopsis thaliana* and *Aegilops longissima*, were determined. The increase in genome size of the two different autopolyploid lines was additive. Taken together, we found no evidence for genome size reduction due to autopolyploidization of studied species.

P0636. Limited genome size variation in four subspecies of *Pinus nigra* s.l.

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Despite the fact that genome size should be constant at species level, many reports of intraspecific variations exist. Thus we carried out "ad hoc" analysis to determine the possible existence of nuclear DNA content variation in European black pine (*Pinus nigra* s.l.), a good model for a such study given its karyological uniformity, morphological polymorphism, broad geographical distribution and ecological plasticity. The panel comprised 18 populations across the natural range of *P. nigra* from Europe including four subspecies: subsp. *nigra*, *salzmanni*, *dalmatica* and *pallasiana*. Mean 1C DNA content was 23.65 pg (± 0.203) assessed by flow cytometry. The coefficients of variation within and between populations did not exceed 2%. We have already reported the existence of significant differences for three Black pine populations. Slight intraspecific variation was also confirmed in the present study dealing with many more populations. There were no relationships between 1C DNA content and taxon affiliation of *P. nigra* subspecies. We conclude that intraspecific variation was not taxonomically important in our case.

P0637. Genome Size in Mediterranean Malvaceae: Karyological, Ecological and Evolutionary Implications.

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This karyological study of subtribe *Malvinae* comprises 33 species from *Althaea*, *Lavatera*, *Malope* and *Malva*, genera displaying their maximum diversity in the Western Mediterranean Basin. The patterns of variation in DNA amounts measured show coherence with other sources of evidence (ITS data, fruit morphology), being of high utility for the systematic study of Mediterranean *Malvinae*. DNA amounts ranged from 2C = 1.25 pg in *Malva parviflora* to 2C = 10.94 pg in *M. alcea*. This means a 8.7-fold overall range of variation. Chromosome numbers vary from 2n = 14 in *L. trimestris*, the only diploid of the group (x = 7), to 112 in *L. cretica*, which corresponds to 16-ploid. Well defined, both evolutionary and ecological trends are observed in the distribution of DNA amounts. Strong differences in DNA amounts among types of life cycles appear. The average DNA amount for ephemerals is 2C = 1.79 pg, less than the average for annuals, 2C = 2.93 pg. This value is similar to the DNA content of facultative perennials, 2C = 2.85 pg. The obligate perennials display the largest value, 2C = 6.32.

P0638. Genome size in *Cirsium* and its relation to natural hybridization

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Nuclear DNA amounts of 12 diploid and one tetraploid taxa and 12 natural interspecific hybrids of *Cirsium* from 102 populations in the Czech Republic, Austria, Slovakia and Hungary were estimated using DAPI and PI flow cytometry. 2C-values of diploid (2n=34) species varied from 2.14 pg in *C. heterophyllum* to 3.60 pg in *C. eriophorum* (1.68-fold difference); the 2C value for the tetraploid *C. vulgare* was 5.54 pg. AT frequency ranged from 48.38 % in *C. eriophorum* to 51.75 % in *C. arvense*. Biennial species of *Cirsium* possessed larger nuclear DNA amounts than their perennial relatives. Genome size was negatively correlated with Ellenberg's indicator values for continentality and moisture and with eastern limits of distribution. C-values positively corresponded with the spinyess (degree of spinosity). The hybrids of diploid species remain diploid, and their DNA content was smaller than the mean of the parents'. Species with smaller genomes produce interspecific hybrids more frequently. Negative correlation is between frequency of natural hybridization and the genome size difference between parental taxa in the most frequently hybridizing *Cirsium* species pairs.

P0639. Karyological study of SE Alpine and Central European taxa of *Luzula* sect. *Luzula*

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Luzula sect. *Luzula* is the most taxonomically diverse group within the genus. It displays a wide range of chromosome numbers due to polyploidization and chromosome fragmentation (agmatoploidy). In several taxa from Slovenia, NE Italy and S Austria genome size (C-value) was measured in root tip cells densitometrically with interphase-peak DNA image cytometry. Chromosome number was determined on the same slides. Since agmatoploid and peculiar partial agmatoploid karyotypes are confined to higher altitudes, a special effort was made to include all the SE Alpine taxa. As expected, C-value proved to be the same in diploid (12 AL: *L. campestris*) and agmatotetraploid (24 BL: *L. cf. sudetica*, *L. cf. divulgata* and 48 CL: *L. sudetica*) taxa and approximately doubled in true tetraploid (24 AL: *L. divulgata*, *L. multiflora*1) and tripled in true hexaploids (36 AL: *L. multiflora*2). C-value of partial agmatoploid (12 AL+24 BL: *L. alpina*) approximately equals the DNA amount in tetraploids. Karyological variability of *L. multiflora* (24 AL and 36 AL) and taxonomic status of Alpine *L. cf. sudetica* and Submediterranean *L. cf. divulgata* require further study.

P0640. Why is it so difficult to obtain high quality AFLP profiles in *Argyranthemum*?

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Argyranthemum (Asteraceae: Anthemideae) is the largest genus endemic to the Atlantic oceanic islands in Macaronesia. Its diversification has been studied by morphometrics as well as crossing experiments, isozymes, cpDNA restriction sites, ITS sequences, and FISH/GISH. All species are diploid (2n=18), suffrutescent, and interfertile. To investigate the genetic structure of two species of reciprocal hybrid origin, *A. lemsii* and *A. sundingii*, and their progenitors, *A. broussonetii* and *A. frutescens*, we tried to develop AFLP fingerprinting markers. Several AFLP protocols were tested. We increased the number of selective bases in one or both of the primers, and used a third restriction enzyme (TE-AFLPs). All protocols gave a multitude of fragments but no high quality AFLP profiles and only a few fragments that could be reliably scored. The genome sizes (1C) of the progenitor species are 7.07 and 7.21 pg, which should be workable genome sizes for normal AFLP protocols. Our AFLP problems might then be related to strongly amplified fragments that represent repetitive, high copy loci or parts of transposable elements.

P0641. Genome size of desiccation-tolerant *Ramonda serbica* and *R. nathaliae* (Gesneriaceae)

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Ramonda serbica and *Ramonda nathaliae* are extremely rare representatives of resurrection plants among Holarctic phanerogamic flora. Both species are endemics of Balkan peninsula and Tertiary relicts. The sample concerns two populations of *R. serbica* (one from Serbia and one from Montenegro) and one population of *R. nathaliae* from Serbia. Nuclear DNA content and GC percentage were assessed by flow cytometry. The 2C DNA values for *R. nathaliae* and *R. serbica* were 2.38 pg and 7.77 pg, respectively. These results were in accordance with different ploidy levels of these species. The base composition for *R. nathaliae* was found to be 42.06% GC. The significant intra-population variation of DNA amount was observed in *R. serbica* from Montenegro. The 2C DNA content ranged from 2C=7.62 pg to 11.31 pg and revealed existence of different ploidy levels between individuals of this population.

P0642. Ploidy level variation in *Lythrum salicaria* L. (Lythraceae) at large spatial scale

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Purple loosestrife (*Lythrum salicaria* L.), a native Eurasian species, belongs among highly invasive angiosperms in North American wetlands. Ploidy level of numerous progenies rising from nearly 600 mother plants sampled at both primary and secondary areas of distribution was screened using flow cytometry. Large cytotype variation (2x, 3x, 4x, and 6x) was found across the native range (76 populations covering 14 European and Middle East countries) DNA-tetraploids prevailed in the area studied, while DNA-diploids and DNA-hexaploids were recorded only in Israel and Turkey, respectively. DNA-triploid progenies occurred in one population from Hungary. Sympatric growth of tetraploid and hexaploid individuals was encountered in Turkey. On the contrary, cytotype uniformity is a typical feature for the non-native American material (77 populations covering 13 states of the U.S.A. and Canada) where only DNA-tetraploids were revealed.

P0643. Genome size variation and evolution among diploid *Gossypium* species

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Although genomes of eukaryotic organisms vary approximately 80,000-fold in size, there is only a 20-fold variation in the number of protein-coding genes. Based on comparisons of orthologous regions among several taxa, it is generally agreed that the majority of this genome size variation can be ascribed to the repetitive fraction. Therefore it is of interest to determine the components of DNA that contribute to genome size variation, and its relevance to organismal fitness and evolution. Although all diploid members of *Gossypium* have a haploid complement of 13 chromosomes, genome sizes range 3.0-fold, from 885 to 2572 Mb per 1C nucleus. This wide range in genome size, in addition to a well-established phylogeny, makes *Gossypium* an excellent system for the study of genome size evolution. Genomic shotgun libraries were constructed from three members of *Gossypium* and one outgroup species. Approximately 0.14% of each haploid genome was sequenced for each of the species. The results suggest that a major fraction of the genome is composed of repetitive *gypsy*-type retrotransposons and that these sequences are responsible for much of the observed genome size variation.

P0644. Genome size variation in the endemic sagebrushes and their allies (*Artemisia*, *Anthemideae*, *Asteraceae*) of North America.

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Genome size of around 30 taxa belonging to the North American sagebrushes and related species (mainly from subgenus *Tridentatae* of the genus *Artemisia*) has been assessed by flow cytometry, including all the most common sagebrushes as well as some narrow endemics. The higher nuclear DNA amount per basic chromosome set of the *Tridentatae* compared with that of the other sections and subgenera of the genus is confirmed. The possibility of finding any connection between genome size data and morphological, physiological or environmental traits of the studied species has been tested. However, little interspecific variation has been found, the most notable being the differences in C-value with taxa of doubtful taxonomic position in the section, namely *A. bigelovii* or *A. pygmaea*. It is suggested that such homogeneity in nuclear DNA amount (together with the high morphological, chemical and karyological affinities) supports the hypotheses of a recent diversification process in this restricted group of the genus. Additionally, low values of intraspecific and intrahybrid nuclear genome size variation have been detected for most taxa.

P0645. Genome size estimation in herbarium vouchers by DAPI flow cytometry

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DAPI flow cytometry was applied for genome size (ploidy level) estimation in herbarium vouchers of vascular plants. Effect of different drying methods, storage conditions, and the age of herbarium vouchers (1-36 months) on peak attributes was tested using model taxon *Vaccinium* subg. *Oxycoccus* comprising four cytotypes (2x, 4x, 5x, 6x). While mode of desiccation had little impact on histogram quality, material stored in deep freezer showed longer lifetime and yielded significantly lower CVs than samples stored at room temperature. Subsequent investigation of 20-month old herbarium vouchers of various vascular plants (pteridophytes, gymnosperms, and angiosperms) gave reproducible signals in 43 out of 60 taxa. In comparison with the fresh material, certain decrease in relative fluorescence intensity (0.2 - 11.7%, mean 4.6%) occurred in most dry samples, being negatively correlated with genome size. Demonstrated feasibility of ploidy estimation in herbarium vouchers further broadens the versatility of flow cytometry and opens new prospects in plant biosystematics.

P0646. Genome size evolution among *Gossypium* species

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Plant genomes range in size over three orders of magnitude. Mechanisms of genome expansion and contraction likely have contributed to this disparity, but the relative role played by each in shaping modern genomes is unclear. Comparative sequencing of large, homologous regions of genomic DNA from organisms that differ in genome size provides an opportunity to reveal mechanisms of genome size change. We present results from an ongoing analysis based on comparative sequencing of homologous BACs in *Gossypium*. Despite the relatively young age of the genus (5-10 mya) and its conserved complement of chromosomes (n = 13) and genes, 1C values for the genus range over 3-fold, from 885 to 2572 Mbp. Aligned BACs from interspecific comparisons suggest that the primary cause of genome size expansion is transposable element proliferation, whereas small scale deletional processes may counterbalance genome growth via mechanisms such as illegitimate recombination. Furthermore, the data indicate that the mechanisms that lead to genome size differences do not affect all genomic constituents equally.

P0647. Appendages matter, again: Phylogenetics and the classification of *Curcuma* L.

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The genus *Curcuma* L. (Zingiberaceae) is an important part of tropical Asian floras and many Asian cultures, yet the identification of the vast majority of species has been fraught with difficulty due to possible hybridization and polyploidization. Here we take a

phylogenetic approach to understand the basic evolutionary pattern of *Curcuma*, using the nuclear ribosomal ITS and chloroplast matK/trnK. A broad representation of taxa was sampled, including morphological and geographic extremes of the genus. Also included are five unusual genera: *Hitchenia glauca*, *Laosanthus graminifolius*, *Smithatris supraneana*, *S. myanmarensis*, *Stahlianthus involucratus*, and *Paracautleya bhatii*. A paraphyletic *Curcuma* including these genera was found, creating a monophyletic 'Curcuma clade', and the generic definition therefore expanded to include them. The three major clades in the phylogeny are used to subdivide the genus for the first time into three subgenera. The significance of hybridization and polyploidy in the genus is discussed.

P0648. Microsatellite markers for *Tragopogon dubius*

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Microsatellite markers are dispersed throughout the nuclear genome and consist of tandemly-duplicated di- or trinucleotide repeats. Because they evolve rapidly, microsatellite loci are useful to compare genetic diversity at the species or population level. We have developed a microsatellite library for *Tragopogon dubius*. This diploid species served as the common parent of the allotetraploid species *T. mirus* and *T. miscellus*, both of which formed multiple times in the Pacific Northwest of the U.S. within the last 80 years. Interrupted and continuous microsatellite loci were examined, with the expectation of higher diversity among alleles with continuous tandem repeats. Using these microsatellites, we compared genetic diversity between North American and Eurasian populations of *T. dubius*. These markers will also be used in the construction of a genetic map for *T. dubius* and will contribute to ongoing work to improve our understanding of recurrent polyploidy using *Tragopogon* as a model.

P0649. Molecular phylogeny and origin of polyploid gorses (*Ulex*, Fabaceae).

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Ulex (Fabaceae) is a small euploid series, which occurs in western Europe, mainly in the Iberian Peninsula. The hexaploid *U. europaeus* subsp. *europaeus* is notorious for being now an invasive weed in different continents, following introductions during the last two centuries. The use of ETS nrDNA, ITS nrDNA and *trnL-trnF* cpDNA data to investigate the evolutionary history of *Ulex* provided insights to elucidate most relationships among the diploid taxa native from either the *atlantic* or the *mediterranean* areas and allowed inference on the origins of the polyploids. *Ulex breoganii* (4x) and *U. gallii* (6x) share a single ETS genotype identical to that of the Atlantic diploid *U. minor*, suggesting their probable autopolyploid origin. *U. argenteus* subsp. *erinaceus* (4x) and *U. argenteus* subsp. *subcericeus* (6x) appear to derive from a common Atlantic ancestor, while *U. latebracteatus* (4x) is most probably deriving from the Mediterranean *U. parviflorus* (2x) genotype. Additivity of ETS sequences from both the Atlantic and the Mediterranean genomes in the invasive hexaploid *U. europaeus* subsp. *europaeus* supports the hypothesis of its recent allopolyploid origin.

P0650. Molecular phylogeny and the role of hybridization in the diversification of *Polygonum* section *Persicaria* (Polygonaceae)

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Polygonum sect. *Persicaria* (Mill.) Meisn. consists of ca. 70 species of weedy herbs found in moist or disturbed areas. Phylogenetic analyses were conducted using nuclear (ITS and *LEAFY* first and second intron and second exon) and chloroplast (*matK* and noncoding regions of *psbA-trnH* and *trnL-F*) DNA sequences from 45 species (10 outgroups). Our ITS trees suggest that sect. *Persicaria* is polyphyletic, with *P. amphibium* linked with sect. *Tovara* and the universally distributed *P. lapathifolium* complex basal. In our combined chloroplast trees the basal lineage is not fully resolved and the *P. lapathifolium* complex is allied with South American species, suggesting cytoplasmic introgression or recent diversification of the South American species. The number of *LEAFY* copies is generally consistent with ploidy level. Not all species share a common *LEAFY* copy, indicating possible lineage sorting, but our gene trees support the hybrid origin of several

species as hypothesized based on incongruent nuclear and chloroplast gene trees.

P0651. Ploidy patterns in the arctic-alpine polyploid *Draba lactea* Adams (Brassicaceae) and its low-ploid relatives

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Ploidal level information is of particular importance in intricate polyploid complexes such as in arctic-alpine *Draba*. Relative DNA content and reference chromosome numbers were determined for the tetra- and hexaploid *D. lactea* and seven of its low-ploid relatives. Absolute DNA content was measured by internally standardised Feulgen densitometry. The plants grouped into diploids (*D. altaica*, *D. fladnizensis*, *D. lonchocarpa*, *D. nivalis*, *D. palanderiana* and *D. subcapitata*; $2n = 16$), tetraploids (*D. lactea*, *D. turczaninowii*; $2n = 32$), hexaploids (*D. lactea*; $2n = 48$), and two odd triploids. *Draba palanderiana*, reported as di-, tetra- and octoploid, was diploid in all investigated accessions. Hexa- and tetraploids were observed in *D. lactea*, in approximately the same ratio (8:1) as previously reported. The ploidal levels of the Central Asian *D. altaica* and *D. turczaninowii* were reported for the first time. In the present study, the DNA amounts of the polyploids increased in direct proportion to ploidal level. In combination with molecular data, these results are pointing to a relatively recent polyploid origin.

P0652. Evolutionary dynamics of nuclear genes and origin of polyploid annual bromes (Poaceae).

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Gene and genome duplications are important evolutionary features of nuclear plant genomes. They represent a critical source of functional diversification and speciation (in both plant and animals). However, these phenomena can be responsible for a series of problem in phylogenetic reconstruction. They may also be misleading in analyses of polyploid species based on molecular data. Indeed, orthology, homeology (in allopolyploid species) and paralogy of the sequences that are used have to be unambiguously ascertained before any analysis. This will be discussed in the context of phylogenetic reconstruction of diploid and polyploid annual *Bromus* species (Poaceae) originating from Eurasia and the Mediterranean region. Data sets from repetitive ribosomal DNA sequences (*ITS*) and low-copy nuclear genes (*Waxy* gene encoding the Granule Bound Starch Synthase I) will be compared.

P0653. Genome origins, recombination, and diversity in a complex polyploid, *Sorghum halepense* (Poaceae)

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The origins and subsequent evolution of the polyploid *Sorghum halepense* (L.) Pers. ($2n = 4x = 40$) have not been well characterized, despite its significance as one of the world's most noxious weeds. We have constructed haplotype networks from several unlinked nuclear loci for individuals of the *S. halepense* species complex. These haplotype networks are used to infer the origins of the genomes of *S. halepense*. Complex patterns are seen in the haplotype networks for individual loci, as well as in comparisons between loci. The evidence supports *Sorghum bicolor* (L.) Moench ($2n = 2x = 20$) as a putative diploid progenitor of *S. halepense*. The contribution of *Sorghum propinquum* (Kunth) Hitch. ($2n = 2x = 20$) is less clear. Introgression, genomic changes accompanying the polyploid event, and tetrasomic inheritance in the polyploid may be responsible for the differing patterns seen between loci. Low levels of nucleotide diversity suggest that *S. halepense* may be of recent origin; however, recombination between the diploid progenitors has created high levels of allelic diversity, which may be contributing to the invasive ability of *S. halepense*.

P0654. Allozyme diversity in the tetraploid endemic *Oxytropis chankaensis* (Fabaceae)

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Oxytropis chankaensis Jurtz. (Fabaceae) is a herbaceous perennial plant endemic of the sand dunes of the west shore of Khanka Lake (Far East of Russia), which is included in the Rare Plant Species Book of Far East of Russia. It is a tetraploid with $2n = 32$. Using starch gel electrophoresis, we examined 16 enzyme systems presumably controlled by 28 loci. Twelve loci were shown to be polymorphic. Banding pattern exhibited no evidence of fixed heterozygosity and showed both balanced and unbalanced heterozygotes at all polymorphic loci, with some individuals exhibiting a pattern consistent with the presence of three or four alleles at a single locus. High levels of genetic variability were found at population level ($P_{95} = 37.1\%$, $A = 1.95$, $H_{ob} = 0.261$). The results support an autotetraploid origin for tetraploid *O. chankaensis*, and the high number of alleles at some loci may be due to repeated polyploidization events. The high values of genetic variation agree with those expected for tetraploids.

P0655. Uncovering the origin of the wheat B genome

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Bread wheat has a polyploid origin from three ancestral genomes. In the past, extensive studies have been carried out to resolve the phylogenetic relationship of genome B to species among the Sitopsis section of Aegilops. In spite of intensive efforts, clear evidence for the B genome origin is still lacking. Identification of the B genome origin may open unexpected possibilities for wheat breeding.

To identify the B genome donor, we characterized 520 accessions of Ae. sect. Sitopsis, using AFLPs at 80 loci. Based on this, 10 lines from each species were selected and compared with B genome from nulli-tetraploid Chinese Spring. We found that some Ae. speltoides are promising B genome donor candidates. To solve this mystery, we extended our analysis by SNP-haplotype studies. Eight loci were amplified for the A and the B genome from 40 T. dicoccoides and six Ae. speltoides lines. For each locus, clearly defined haplotypes were identified. Comparing nucleotide diversity and haplotype structure it is now evident that the B genome of modern wheats arose from an apparently outbreeding Ae. speltoides line, the ancient allelic diversity of which is conserved in modern wheats.

P0656. Allopolyploid speciation in *Senecio*: changes to transcriptome in wild and synthetic hybrids.

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Hybridisation between plant species is one of the best-known mechanisms by which abrupt speciation can occur. The genus *Senecio* provides examples of both homoploid and allopolyploid speciation, though we focus here on the latter. Since its introduction to the UK in the 18th Century, the invasive species *Senecio squalidus* (diploid) has hybridised on at least two occasions to the native *S. vulgaris* (tetraploid) to produce the sterile triploid *S. x baxteri*. This has then undergone a chromosome doubling event to produce the fertile allohexaploid *S. cambrensis*. As an example of recent speciation, *S. cambrensis* is a valuable model for studying changes to gene expression resulting from hybridisation and polyploidy. Here, we describe the construction and use of custom cDNA microarrays from the *Senecio* taxa involved in the allopolyploid formation of *S. cambrensis* to study gene expression between the hybrid and parental taxa. We describe dramatic changes to the transcriptome of the hybrids, with evidence from resynthesised allopolyploids indicating that such changes occur rapidly following hybrid formation.

P0657. Simple technique for determination of ploidy level using chloroplast number in stomatal guard cells of *Trifolium* plants

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Trifolium is a major crop plant. Determination of ploidy level is required for studying interspecific relationships, genetic studying of

transgenic plants and plant breeding programmes. All species of this genus have small chromosomes. Therefore an efficient alternative ploidy determination technique is required for this group of plants. The relationship between ploidy level and the number of chloroplasts in stomatal guard cells, was studied in 24 populations of 8 species of *Trifolium*. Three randomly selected middle leaves of greenhouse grown plants were used for chloroplast counting in twenty pairs of stomatal guard cells per each leaf. Chromosome number were also determined in root tip of studied plants. The ploidy level was highly correlated with the number of chloroplasts in stomatal guard cells. Number of chloroplasts in tetraploid population of *T. fragiferum* was approximately twice as many diploid species: *T. spumosum*, *T. campestre* and *T. physodes*. These results suggested that counting chloroplast number in the leaf stomatal guard cells is an efficient alternative technique for determination of ploidy level in the genus *Trifolium*.

P0658. The copy numbers of the *Leafy/Floricaula* gene in the family Chenopodiaceae

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The *LEAFY/FLORICAULA* (*LFY/FLO*) gene is a key regulator responsible for the transition from vegetative to reproductive development in angiosperms. A single copy of *LFY/FLO* is supposed to exist in diploid plant species. In contrast, polyploids contain several copies of this gene, some of them with modified function. As *LFY/FLO* copy number may reflect ancient polyploidisation events, it does not always correspond to the currently estimated ploidy level.

We analysed the copy number of *LFY/FLO* in 14 representatives of the family Chenopodiaceae, focusing on *Chenopodium* and *Atriplex*. We applied Southern hybridisation of digested genomic DNA with digoxigenin labelled *LFY/FLO* probe. In some species, the number of *LFY/FLO* copies did not correspond to the ploidy level. We sequenced the second intron and its flanking regions and discussed the nature of additional copies. We have performed the comparative analysis of partial *LFY/FLO* sequences in the phylogenetic context of Chenopodiaceae.

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P0659. *Allium oleraceum* L. polyploid complex in the Czech Republic: geographical and ecological pattern

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Allium oleraceum L. represents polyploid complex ($2n = 3x-6x$) of common European geophytes, probably of allopolyploid origin. We examined the population frequencies and distribution of cytotypes in the area of the Czech Republic. Ploidy was inferred from estimates of DNA contents, using flow cytometry, for 4347 plants sampled from 327 populations. We further tested for habitat differentiation among cytotypes by comparing many environmental variables of their respective habitats. Across the entire sampling area, pentaploids were most abundant (53% of individuals) while hexaploids and tetraploids were least abundant (33% and 14%, respectively). We did not find any triploid plant. Most populations contained only one cytotype (77%), 24% contained two and 1% had a three cytotypes. Populations with two cytotypes occurred in all possible cytotype combinations. We found significant differences in geographical pattern among cytotypes. Tetraploids and hexaploids showed partially vicariant pattern of distribution while pentaploids were distributed evenly over the whole study area. The results provide evidence for habitat differentiation among cytotypes.

P0660. Molecular phylogenetic analysis of *Oryza* L. (Poaceae) and its consequences for historical biogeography, cytogenetics and morphological evolution.

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Oryza L. is a small, pantropical grass genus with species generally occurring in flooded grasslands. The genus comprises 23 species with a skewed distribution among infrageneric taxa and ten genomic groups (determined by meiotic chromosome behavior in

hybrids): A, B, C, E, F, G, BC, CD and HJ. Both parsimony and maximum-likelihood phylogenetic analyses were applied, based on chloroplast DNA *trnTLF* noncoding sequences. The phylogenies showed that the small complexes are basal and the large apical complexes are of relatively recent origin, with many poorly differentiated species displaying the ability for rapid intercontinental dispersal. Both the diploid and tetraploid genomes CD and HJ are monophyletic, but the Asian and African BC species seem to have originated from distinct polyploidization events. The Neotropical CD genome may have evolved anagenetically from the African BC. Spikelet morphology assessed from center-to-outline distances separated by constant angles seems to have diverged in an approximately linear way in relation to phylogenetic distance, except for the mainly insular species *O. minuta*, whose spikelets are smaller than predicted.

P0661. DNA rearrangement and progressive diploidization of the paleopolyploid maize genome

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Sequence analyses of orthologous regions from several cereal genomes have indicated the nature and rates of genome change in these lineages. Since the origin of maize from a tetraploid formed less than 12 mya, ~25% of the tetraploid's genes have been lost, mostly through a progressive accumulation of small deletions. The gene losses usually have not removed both orthologues, so that the number of gene families has not changed dramatically. Gene losses appear to be equally likely from both "homoeologues" in most regions, suggesting that epigenetic silencing of long chromosomal stretches on one homoeologue did not commonly occur after polyploidization. Recent tandem gene amplifications and deletions are observed, many associated with unequal recombination. In addition, transposable elements (TEs) have acquired, transposed and thereby amplified numerous genes or gene fragments. The regions between genes show almost no conservation between the two maize homoeologues, sorghum and rice. This is largely because intergene TE amplification and subsequent removal by unequal homologous and illegitimate recombination make these regions exceptionally dynamic.

P0662. A comparative study of chromosome morphology among the some cultivars of *Cicer arietinum* L.

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Eleven cultivars (desi and kabuli types) were subjected to karyotype analysis for their potential use in determination of genetic relationships in chickpea cultivars. Somatic chromosome number $2n = 16$ confirmed in all the cultivars. Within cultivars interchromosomal size variation was observed to be quite large in some cultivars but not in the remaining rest cultivars. Significant differences in haploid genome length among the cultivars were observed (18-25 μ). The cultivars were classified in two groups based on karyotypic similarity, with the first group comprising intercrossable, while the remaining cultivars forming second group. The first group are also genetically close to each other as deduced by other Morphological, Biochemical and DNA based studies circumstantial evidence has lead to the speculation that perhaps karyotypic similarity and intervariety crossability are positively related to each other.

P0663. Karyological and taxonomic notes on *Odontites vernus* group (Scrophulariaceae s.l., tribu Rhinanthae) in the Iberian Peninsula.

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The *Odontites vernus* group is the most widely distributed and probably the most complex from a taxonomical point of view within the genus *Odontites* Ludwig. According to the traditional treatments, it is represented in the Iberian Peninsula for two taxa: *O. vulgaris* Moench, $2n=20$, and *O. vernus* (Bellardi) Dumort., $2n=40$. With the purpose of clarifying the taxonomical status of both species in the Iberian Peninsula, morphological and karyological studies from Iberian material were made. The most repeated ploidy level in the about 50 populations studied is the tetraploid level. Recognizing the individuals of both ploidy levels by

morphological characters does not seem to be possible according to the results obtained from this study. Neither the geographical distribution of the morphological variants nor that of the ploidy levels support the taxa separation. It is possible that both taxa behave here like cryptospecies or that, as a whole, it is a polyploid complex with two ploidy levels ($2n=20,40$); in this case, *O. vernus* should be considered as a unique taxon in the Iberian Peninsula.

P0664. Comparative assessment of induced mutants from *Solanum macrocarpon* L.

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Three mutants (M1, M2, M3) consistently expressed cytological and morphological variations among eight seedlings growth lines treated with 0.6% aqueous solution of colchicine. The five others resumed normal growth after the tenth leaf stage. The mutants experienced delayed germination (8-12 days) and tumour swellings, tip twisted or rolled radicles. M1 and M2 plants had three cotyledons while M3 and controls had two cotyledons each. Leaves were smaller and slightly folding in the mutants. Mutants were procumbent to prostrate with short and fewer internodes than erect control plants. Epidermal cells were extensively wavy in M3 plants, stomata characterised by one guard cell in M1 and closed pores in M2. M2 plants did not produced flower while M1 and M3 produced fewer flowers than controls. M1 contained fewer seeds while fruit ripening was delayed in M3. M1, M3 and control plants exhibited diploid chromosome complement ($2n$) of 24 but doubled in M2. M1 and M3 showed somatic instability, $2n = 28, 32$ and 42. Meiotic irregularities in M1 and M3 include clumps, laggards and bridges.

P0665. Karyotype Analysis of Chilean Taxa of *Alstroemeria* L. (Alstroemeriaceae)

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The Chilean taxa of *Alstroemeria* L. are already profoundly described by classical systematic methods, but relationship is not yet completely resolved. Morphometric and C-banding analysis were applied and proved to be useful for identification of individual chromosomes and species-specific characteristics. Differences in chromosome size are observed on inter- and infraspecific level. Despite of variation in size, chromosomes can be grouped into a typical karyotype with a formula of $2n=16: 1 (m) + 3 (m-sm) + 4 (st-t)$, valid for more than 20 taxa (species and subspecies) examined. Our data show that one chromosome is conserved while others are submitted to the dynamic process of rearrangement during speciation. Additionally FISH-analysis was applied for physical mapping of rDNA sequences (18S-5,8S-28S). The relationship of four Chilean taxa, *A. ligtu* L. (three subspecies) and *A. pulchra* Sims, based on systematic characters, is now confirmed by cytogenetic methods and discussed in detail.

P0666. Contribution to the Cytotaxonomy of the Georgian Flora

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Cytological research of high mountain species of Caucasus was made. Descriptions of the karyotypes and chromosomes number of 100 species (40 endemics and subendemics) were analyzed. It was shown, that these species belonged to the mono- and oligotypic genera or sections: *Brunnera macrophylla* $2n=12$; *Sobolewskaia caucasica* $2n=16$; *Cladochaeta candissima* $2n=18$; *Primula farinifolia* $2n=18$; *P. bayernii* $2n=22$; *Merendera raddeana* $2n=18$; *Colchicum szovitsii* $2n=18$; *C. bifolium* $2n=18$; *C. zangezorum* $2n=18$; *C. woronowii* $2n=42,48$; *C. speciosum* $2n = 38$; *Gagea joannis* $2n=24$; *G. sulfurea* $2n=72$; *Crocus suvorovianus* $2n=8$; *Colpodium versicolor* $2n=4$; *Zingeria trichopoda* $2n=8$. The subnival belt vegetation spreading in the upper limit has been investigated. The origin and differentiation of the subnival floristic complexes were analyzed. The diploids makes up 76%, polyploidy - 24%. Subnival belt contains an ancient oreophits. Phylogeny for the major species based on the cytotaxonomical investigation is discussed.

P0667. A karyosystematic study of some endemic, rare and interesting weeds of Crete (Greece)P. Bareka¹, G. Kamari¹, N. Turland², D. Phitos¹;¹Botanical Institute, Patras, Greece, ²Missouri Botanical Garden, Saint Louis, MO, United States.

In Spring of 2003, a research project entitled "Threatened weeds in the traditional agriculture of Crete", financially supported by the National Geographic Society, took place. The project was a collaboration between the Missouri Botanical Garden, St. Louis U.S.A. (MO) and the Botanical Institute of the University of Patras, Greece (UPA). The goal of this project was to carry out a thorough floristic survey of weeds in sites of traditional agriculture of the South Aegean island of Crete, Greece. During this project, living material was also collected for cytological investigation.

As preliminary results of this karyosystematic study, the chromosome number, karyotype morphology and geographical distribution of some endemic, rare and interesting weeds of Crete are presented here. Further issues of taxonomy, phytogeography and conservation of some Cretan weeds are briefly discussed.

P0668. Strasburgeria robusta (Strasburgeriaceae) survives as a high-polyploid species in New CaledoniaK. Oginuma¹, J. Munzinger², H. Tobe³;¹Human Life & Environmental Science, Kochi, Japan, ²IRD BP A5, Noumea, New Caledonia, ³Graduate School of Science, Kyoto, Japan.

Strasburgeria robusta, the only species of the family of its own, is a tree of 3-15 m tall that grows at elevation of (300-)700-1200 m, mainly in Nothofagus and/or Araucaria humid forest. It is strictly located on ultramafic soil, in the south part of the main island in New Caledonia. Recently Strasburgeriaceae are placed in rosids (APG II 2003), but its relationship is still uncertain. Morphological and vegetative anatomical characters are relatively well known about *S. robusta*, but its cytology is not known yet. We investigated its somatic chromosome number using cells collected from young leaves and flowers buds, and found that the species has as many as about 500 chromosomes. To our knowledge, $2n = ca. 500$ represents the highest number in angiosperms except for several particular species of *Crepis*, *Kalanchoe*, *Spinach*, etc. Although the base number is unclear, there is no doubt that such a chromosome number represents an extremely high-polyploid. While *Strasburgeria* probably had lost its relatives at lower ploidy levels in its history, its descendant has survived only as the extremely high-polyploid adapted to nickel-rich soils in New Caledonia.

P0669. Karyosystematics and molecular phylogeny of TrilliaceaeE. O. Punina, E. M. Machs, E. S. Kim, Y. A. Myakoshina, V. S. Chupov, A. V. Rodionov;
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All species of Trilliaceae carry giant chromosomes, the mean size of which is about 9 Mbp, that is 3 times more than all human genome. Karyotypes of Trilliaceae have revealed high interspecies variability of heterochromatin banding patterns. We have studied the Q- and CMA-chromosome banding patterns of *Paris incompleta*, *P. quadrifolia*, *Daiswa hajnanensis*, *Trillium recurvatum*, *T. erectum*, *T. grandiflorum*, *T. tschonoskii* and *T. camschatcense*. Then we compare p-distance between these species through ITS1-5.8S-ITS2 sequence analysis. It was shown that all *Paris* chromosomes have multiple small AT-rich heterochromatic bands. On the other hand, there are large both AT- and GC-enriched heterochromatic bands on a part of chromosomes of *Daiswa* karyotype. The chromosomes of all *Trillium* species, with exception of *T. tschonoskii*, carry large AT-enriched heterochromatic bands. Our results of ITS1 and ITS2 sequences analysis support the position of *T. tschonoskii* on the branch of *Paris* species in spite of morphology analysis and chloroplast gene matK sequence of this species are clustered *T. tschonoskii* with *T. camschatcense*. Grants RFBR 03-04-49477 and 02-04-49896.

P0670. Citotaxonomy of the subtribe Lychnophorinae (Vernoniaeae: Asteraceae)E. R. Forni-Martins^{1,2}, M. E. Mansanares^{3,4}, J. Semir¹, A. L. L. Vanzela⁵;¹Universidade Estadual de Campinas, Campinas, SP, Brazil, ²CNPq, Brasília, Brazil, ³Curso de Pós-graduação em Biologia Vegetal, Universidade Estadual de Campinas, Campinas, SP, Brazil, ⁴FAPESP,São Paulo, Brazil, ⁵Universidade Estadual de Londrina, Londrina, PR, Brazil.

Lychnophorinae is composed by 9 genera, most of them endemic to the Brazilian *campos rupestres*. In the genus *Lychnophora* there is disagreement between different authors regarding the limit and number of species. The difference is based on sinonimization and transference of several species to related genera. There are also difficulties in the delimitation of other genera (*Minasia*, *Proteopsis* and *Heterocoma*). Aiming a better understanding of the taxonomy of the group as a whole, chromosome numbers of about 49 species were determined. The numbers $2n=34$, 36 or 38 were distributed among species of several sections of *Lychnophora* and also closely related genera. Chromosomes size (1.0 to 2.58 μ m) and morphology (mainly metacentric, with some submetacentric) were analyzed in 7 species and showed relative constancy. The use of FISH, with 45S rDNA probe, have demonstrated great diversity, with 4 to 10 hybridization sites among 16 species. Although karyotype characters can't be used to segregate genera and sections of *Lychnophora*, they are very useful for the differentiation between some species, which taxonomic limits have been previously questioned.

P0671. Origin and evolution of sweet potato (*Ipomoea batatas*) and its wild relativesS. Srisuwan, D. Sihachakr, S. Siljak-Yakovlev;
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Sweet potato (*Ipomoea batatas*), a hexaploid species ($2n=6x=90$), is an important food crop. The origin of its polyploidy and the genome evolution are still being discussed. The relationship among closely related species, or diploid ($2n=2x=30$) parental candidates (*I. trifida*, *I. triloba*, *I. leucantha*, *I. tiliacea* and *I. setosa*) has been investigated by using molecular markers. However, molecular-cytogenetic analysis is lacking through those investigations. In this study, the new data concerning karyotype and genome size of those species are reported. The genome size of hexaploid *I. batatas* (3.45 pg) was about three times as high as that of diploid species (1.17pg). The fluorochrome banding was used to determine GC rich DNA regions, the fluorescence in situ hybridization (FISH), the number and position of 18S-26S and 5S rDNA loci, and the silver staining, to study NORs activity. Those chromosome markers were found effective to distinguish the karyotype of 5 diploid species and two varieties of *I. batatas*. Moreover, the close relationship between *I. trifida* and *I. batatas* has been demonstrated upon analysis of molecular-cytogenetic characteristics.

P0672. Characterizing polyploidy in the Caribbean Consolea Lem. (Cactaceae): chromosomes, cytomixis and pollen grain size.V. Negron-Ortiz^{1,2}, L. I. Strittmatter¹;¹Miami University, Oxford, OH, United States, ²Smithsonian, Botany Department, Washington, DC, United States.

Chromosome counts within the Cactaceae have indicated the presence of euploid and polyploid species, and it has been suggested that both

polyploidy and hybridization events have led to speciation in this family. We studied the ploidy level and chromosome number of *Consolea*, a genus of nine species endemic to the Caribbean. *Consolea* is sub-dioecious with male, female and, in a few species, hermaphroditic plants. Mitotic

chromosome counts on root tips were determined for eight species, resulting in 6x, 7x and 9x ploidy levels. Neither diploids ($2x=22$) nor dodecaploids were found as formerly reported. Flow cytometry is being used to confirm the chromosome counts, and test for presence of sex chromosomes. Cytomixis and variable sized pollen grains were noticed in

several species. Cytomixis, the migration of nuclear material through cytoplasmic channels, has often been observed during microsporogenesis of genetically unbalanced, and seldom, diploid plants.

We detected cytomixis in male-fertile and male-sterile anthers of microspore mother cells, and between a tapetal cell and a microspore

mother cell. Pollen sizes will be compared among the polyploid species.

P0673. A taxonomic study in section *Sacculiferum* of *Allium* from KoreaB. U. Oh¹, C. G. Jang²;¹School of Life Sciences, Chungbuk National University, Cheongju, Republic of Korea, ²Biotechnology Research Institute, Cheongju, Republic of Korea.

The sect. *Sacculiferum* is one of the members of subgenus *Rhizirideum* that is restricted in Northeastern Asia. This section has morphological characters such as ovate bulb, longer filaments than perianth, purplish flower, and subglobular umbel in common. There are known 9 taxa of the section with 4 endemics in Korea. Although each taxon of the section is mainly delimited by leaf shape, growing pattern, the size of umbel, the shape of perianth, and chromosome number, taxonomy of this section is not an easy matter. The main problems of taxonomy is the classification between *A. sacculiferum* and *thunbergii* complex. The most part of members in this section have $2n = 16$ ($x = 8$) in their basic chromosome number. As *A. sacculiferum* and *pseudojaponicum* were tetraploid of $2n = 32$, they were well separated from other taxa. The karyotype of *A. thunbergii* complex was little bit different features from other taxa of the section. The sect. *Sacculiferum* was defined 3 different groups with cytological data. Giemsa C-banding data will be also used to investigate relationships within the section and discriminate the taxa in *A. thunbergii* complex.

P0674. A tale of two parasitic lianas :*Cassytha* and *Cuscuta* [The comparative karyology]

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The karyotype study of two native, parasitic lianas, *Cassytha filiformis* L. ($2n = 24$) and *Cuscuta reflexa* Roxb. ($2n = 32$) revealed five types of chromosome groups based on centromeric index (F%). Type A: Chromosomes C.12.5 μ m long with two constrictions, one nearly terminal (F% = 8%) and the other nearly submedian (F% = 36%). Type B: Chromosomes (6.6 - 24.2 μ m) with nearly median constriction (F% = 38.8 - 47.9%). Type C: Chromosomes (3.4 - 16.1 μ m) with nearly submedian constriction (F% = 22.8 - 37.4%). Type D: Chromosomes (9.3 - 9.7 μ m) with two constrictions, one nearly subterminal (F% = 14.8%) and the other nearly submedian (F% = 37%). Type E: Chromosomes (7.2 - 14.6 μ m) with nearly subterminal (F% = 18.3%) to nearly terminal (F% = 6.5%) constriction. The population of *Cassytha* revealed symmetric karyotype with range of chromosome length 6.3 - 12.6 μ m, disparity index, 33.33% and karyotype formula A2B4C18 while the population of *Cuscuta* revealed asymmetric karyotype with range of chromosome length 3.4 - 24.2 μ m, disparity index, 84%, and karyotype formula B14C6D2E10. The meiosis of *Cuscuta* revealed pericentric inversion bridge and secondary origin of long chromosome.

P0675. Chromosome numbers in selected species of *Hypericum* L. in Lithuania

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Hypericum perforatum and *H. maculatum* are known as species producing a wide range of pharmaceutically important secondary metabolites. As indicated by previous studies, the wild populations of the species is rich primary source of genotypes for the plant breeding and cultivation. Seeds were collected from the natural habitats and field collection and germinated in Petri dishes on wet filter papers. Seedlings with the roots of ca. 5 mm in length were chosen for the karyological studies. These studies confirmed that *H. perforatum* is characterized by a tetraploid chromosome number ($2n = 4x = 32$), but morphologically inconspicuous hexaploids ($2n = 6x = 48$) also occur. Variability of the number of chromosomes in this species is associated with specific way of its reproduction, a facultative apomixis. The main reasons for the selection and breeding of the polyploids might be higher contents of secondary metabolites. *H. maculatum* is a diploid ($2n = 2x = 16$) and it reproduces sexually. The chromosome counts reported here also facilitates distinguishing between species and their natural hybrids.

P0676. Characterization and chromosome location of repetitive DNA in *Anemone hortensis* L.

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Karyotype of the Mediterranean species *Anemone hortensis* L. consists of three acrocentrics (chromosomes 1, 2 and 3), one meta-submetacentric (chromosome 4) and four metacentrics (chromosomes 5, 6, 7 and 8). In *A. hortensis*, large AT-rich heterochromatic bands are located intercalary and terminally on long arm of all acrocentrics and terminally on chromosomes 4 and 5. In this study, we report the nucleotide sequences and chromosomal organization of repetitive elements. The tandem repeats isolated after digestion of genomic DNA with *EcoRV* (JME) and *HindIII* (JMH2) were 567 bp and 743 bp long, respectively. The JME satellite sequence family was located in all intercalary DAPI bands, while the JMH2 satellite sequence family coincided to terminal DAPI bands. After digestion of genomic DNA with *HindIII*, 945 bp long JMH1 repeat sequence was isolated. FISH revealed dispersed organization of JMH1 on all chromosomes of *A. hortensis*. JMH1 exhibited partial homology to the *Ty3/gypsy*-like retroelement and has to be confirmed as a part of a retrotransposon.

P0677. Caryological Study of Some High-Mountain Rare and Endemic Species of the CaucasusT. Gviniashvili¹, R. Gagnidze^{1,2};¹N. Ketskhoveli Institute of Botany, Tbilisi, Georgia, ²I. Javakhishvili Tbilisi State University, Tbilisi, Georgia.

The aim of the study was to investigate caryology of rare and endemic species of the Caucasus high mountains. For number of years the authors studied the main components of floristic complexes and different taxons of the Caucasus high mountain flora. The methods used in the study were taxonomical, areological, ecotopological and caryogeographical. Endemism is an especially common phenomenon for the Caucasus high mountain rocky-scrub-cobble ecotypes. Most of the species of these ecotypes are appear to be diploids. The results of our study confirm that diploid species of the Caucasus high mountain flora are autochthonous. They are of the relatively ancient age. However, polyploids, species with high chromosome numbers, are the result of a further selection process. These species overcame different inconvenient climatic barriers during the long time of the evolution and thus, they might be considered as palaeopolyploids. One of the most important factors in the evolution of the Caucasus endemics is geographical isolation.

P0678. Chromosome number and meiotic behaviour studies in *Hoffmannseggella* H.G.Jones (Laeliinae, Orchidaceae)J. Y. Costa^{1,2}, E. R. Forni-Martins^{3,4};¹Curso de Pós Graduação em Biologia Vegetal, UNICAMP, Campinas, Brazil, ²FAPESP, São Paulo, Brazil, ³Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil, ⁴CNPq, Brasília, Brazil.

Hoffmannseggella is a Brazilian endemic genus of rupicolous orchids, previously assigned to the *Parviflorae* section of *Laelia*, distributed all over the Espinhaço Range, mainly in Southeast region. We analysed mitotic and meiotic chromosome numbers of 9 *Hoffmannseggella* species: 6 presented $n=20/2n=40$ and 2 presented $2n=80$ with aneusomatic root tissues. *H. rupestris*, one of the polyploid species, presented two cytotypes ($2n=40$ and $2n=80$). A new species, found in a parallel study, presented $2n=44$ chromosomes, being the first documentation of a *Hoffmannseggella* species originated by aneuploidy. Meiotic abnormalities are observed in several species, like one extra bivalent, tetravalents, chromosome laggards in anaphase and tetrads with micronucleous. However, all species presented high normality of tetrads (86.4 - 100%). Polyploidy, aneusomaty and most of the meiotic abnormalities were found in species distributed on the Diamantina Plateau, one specific region of the Espinhaço Range, where species usually presented sympatry and flowering period overlapping, suggesting that this region could represent a hot spot for speciation inside the genus.

P0679. Chromosome analysis of the genus *Skimmia* (Rutaceae) in East AsiaT. Fukuda¹, H. Nagamasu²;¹Graduate School of Science, Kyoto University, Kyoto, Japan, ²Kyoto University Museum, Kyoto, Japan.

Chromosome analysis was performed for three species of *Skimmia* in East Asia; *S. japonica*, *S. reevesiana* and *S. arisanensis*. Chromosome counts revealed that *S. japonica* was diploid ($2n=30$) over its entire distribution, and the other two species were tetraploid ($2n=60$). This result does not contradict the report of Johnson & Taylor (1989). All species had five large pairs and 10 rather small pairs of chromosomes. *S. japonica* var. *lutchuensis* in Ryukyu, which grows as large trees, was also found to be diploid, which indicates that plant size and form are not related to ploidy level in *S. japonica*. Karyotype analysis revealed some differences, which can be represented as four karyotypic formulae. In *S. japonica* three karyotypes were found. A-type had the widest distribution, but the chromosome pair with the lowest long/short arm length ratio showed geographical variation; lower than 1.2 in Honshu and further north, and more than 1.2 for southern Kyushu to Taiwan. C-type and D-type were found only in Yaku Island and Taiwan respectively. Karyotype of the two tetraploid species (B-type) differ from *S. japonica* in having only one subterminal pair.

P0680. Cytotaxonomy of some species of *Cousinia* (Asteraceae) from Iran

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Cousinia with a total of c. 662 species, of which 379 are endemic within the Flora Iranica area, is remarkable in various respects. In the recent years some new species or new records of *Cousinia* are added to the Flora of Iran. The generic distribution area of *Cousinia* is nearly identical with the Irano-Turanian region. In this study original observations on meiotic cells in twenty eight species of the genus *Cousinia* are reported. Of these, the chromosome numbers for sixteen endemic species viz, *C. aitchisonii* C. Winkl. ($n=13$), *C. alexenkoana* Bornm. ($n=13$), *C. behboudiana* Rech.f. & Esfand. ($n=12$), *C. boissier* Buhse ($2n=24$), *C. canolleana* Jaub. & Spach. ($n=9$), *C. gmelini* C. Winkl. ($n=12$), *C. irritans* Rech.f. ($n=13$), *C. kerejensis* Bronn. & Gauba. ($n=12$), *C. lasiandra* Bunge ($n=13$), *C. meshhedensis* Bronn. & Rech. f. ($n=13$), *C. pichlorina* Bronn. & Rech. f. ($n=12$), *C. pinarcephala* Boiss. ($n=12$), *C. raphiostegia* Rech.f. ($n=13$), *C. trachylepis* Bunge ($n=13$), *C. turkmenorum* Bornm. ($n=11$), *C. wilhelminae* Rech. f. are reported for the first time. Twelve species provide confirmation of scarce or disputable previous data.

P0681. Giemsa C-banding and karyological studies in species of *Rhinopetalum* (Liliaceae)

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C-band patterns and karyology are described for three Old World *Rhinopetalum* species. All species have a similar basic karyotype ($n=12$), consisting of large symmetric (m, sm) and smaller asymmetric (t, st) chromosomes, but C-bands differ between them. The bands are rather few, located at intercalary, telomeric, centromeric and rarely secondary constriction regions. The genus *Rhinopetalum*, is comparatively richer in heterochromatin. The patterns are characterized by the occurrence of thick telomeric/subtelomeric heteromorphic bands in the second pair of m-chromosomes. Presence of a distinct centromeric band in the short arms of these m-chromosomes in *Rh. bucharicum* discriminate it from the two allied species. A certain level of banding heteromorphy was observed mostly in term of bands size. It is obvious from this study that diversity exists between individual species studied both in the dispersion and quantity of detectable heterochromatin and chromosome morphology. Further aspects of banding patterns, band heteromorphy, chromocenters, equilocal position of bands and the role of C-banding in taxonomy are discussed.

P0682. A critical review and a new proposal of karyotype asymmetry indices

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In the literature nine different methods (the karyotype classification of Stebbins, the TF%, the Rec, the As K%, the Si, the A₁, the A₂, the AsI and the DI) of evaluation of the karyotype asymmetry were found, that are used to delineate the the direction of phylogenetic changes among and within different taxa. Expanded investigation of these nine methods reveals that qualitative classification of Stebbins is a less sensible and only one parameter, the A₂ index, correctly describes the variation of chromosome length in a complement. A new asymmetry index (the AI) is proposed to measure the karyotype asymmetry and a new parameter, the CV_{CI}, is offered, that precisely assess relative variation of centromeric position. The AI index, the CV_{CI} and the CV_{CL} (=A₂ index) have the potential to display even minor karyotypic variations. Thus, these three indices together increase precision of results in comparison with other existing methods. All of them can be used to test evolutionary hypotheses and may be included in multivariate analysis. All this has important consequences in the interpretation of results of karyological studies, especially in evolutionary context.

P0683. FISH of tandemly repeated DNA sequences and RAPD analysis for characterization of geographically isolated populations in five species of *Alstroemeria* (Chile)O. Schrader¹, C. M. Baeza², H. Budahn¹;¹Federal Centre for Breeding Research on Cultivated Plants, Institute of Horticultural Crops, Quedlinburg, Germany, ²Departamento de Botánica, Universidad de Concepción, Concepción, Chile.

In fifteen geographically isolated populations of five species of *Alstroemeria* (*A. aurea*, *A. ligtu*, *A. hookeri*, *A. pelegrina* and *A. presliana*), collected in Chile, were investigated the karyotypes and variation of RAPD markers. Tandemly repeated DNA sequences - 5S and 18/25S rDNA genes and the sequence A001-I (De Jeu et al. 1997) were used in characterizations of karyotypes carried out by fluorescence *in situ* hybridization (FISH). For measurements of chromosomes was used 10 somatic metaphases per population. Differences in 550 RAPD marker bands were used for characterization of populations creating an index of similarity. The FISH with all three DNA probes had shown a high degree of polymorphism between and sometimes also within the populations of *A. aurea*, *A. hookeri* and *A. ligtu*. Furthermore, comparing the 4 populations of *A. hookeri* between themselves, the population 4181 was different in their arm length of chromosome 3, showing a higher difference in relationship to the other 3 populations. In the same comparison the RAPD markers (index of similarity) showed a lower value. The possible evolutionary mechanisms providing these polymorphisms were discussed.

P0684. Structure and evolution of centromere satellite sequences in *Arabidopsis halleri* spp. *gemmifera*A. Kawabe¹, S. Nasuda²;¹Institute of Evolutionary Biology, Edinburgh, Scotland, United Kingdom, ²Kyoto University, Kyoto, Japan.

Centromeric tandem repetitive sequences were isolated from *Arabidopsis halleri* ssp. *gemmifera*. Two novel repeats families, pAge1 and pAge2 were found. The repeat units are about 180 bp and are arranged in head to tail organization. The repeats are similar to the pAL1 180-bp repeat of *A. thaliana* and pAa of *A. arenosa*. *A. gemmifera* also has the sequences of the pAa family. Sequence divergence between *A. gemmifera* and *A. thaliana* is about 30%, about twice that estimated for protein coding gene regions. One region including Box B showed high conservation among the different centromeric repeat families, while several regions differ mainly between the three families. The sequences from different species could not be distinguished. FISH analysis showed that the three 180-bp repeat families have chromosome-specific locations, though some chromosomes have two repeat families. The chromosome specificity of the three repeat families differs among *A. gemmifera* strains, and in a related species *A. lyrata* ssp. *kawasakiana*.

P0685. Comparative karyotype analysis in *Chaetanthera chilensis* (Willd.) DC. and *Chaetanthera ciliata* Ruiz et Pavón (Asteraceae) by double fluorescence *in situ* hybridizationC. M. Baeza¹, O. Schrader²;¹University of Concepción, Department of Botany, Concepción, Chile, ²Federal Centre for Breeding Research on Cultivated Plants, Institute of Horticultural Crops, Quedlinburg, Germany.

Karyotypes of *Chaetanthera ciliata* and *C. chilensis* from Chile were examined using the location of the 5S and 18S/25S rDNA gene sequences. Signals of 5S and 18/25S rDNA are seen in 4 and 5 of the 11 chromosome pairs in *C. ciliata* and *C. chilensis*, respectively. The reported karyotypes are symmetric with small chromosomes and a great similarity among them. Chromosome asymmetry indexes (AsI % and A_i) were similar for both species. This report is the first on chromosome number and karyotype analysis of *Chaetanthera ciliata* and *C. chilensis*. One of the remarkable differences between the karyotypes of *C. ciliata* and *C. chilensis* is that *C. ciliata* has only metacentric chromosomes, whereas *C. chilensis* has a submetacentric chromosome pair. These differences in the karyotype suggest that structural changes or rearrangements may have contributed to the diversification of the genus. Using the FISH technique, notable differences can be observed with respect to the position and number of rDNA sequences depending on the chromosome.

P0686. Genome organization of some *Iris* species assessed by molecular cytogeneticsS. Sijak-Yakovlev¹, F. Bogunic², E. Muratovic³, M. E. Solic⁴, D. Pavlovic⁵, S. Medjedovic²;¹Ecologie, Systématique, Evolution, UMR CNRS 8079, Orsay, France, ²Faculty of Forestry, Sarajevo, Bosnia and Herzegovina, ³Faculty of Sciences, Sarajevo, Bosnia and Herzegovina, ⁴Institute Mountain and Sea, Makarska, Croatia, ⁵Faculty of Science, Kragujevac, Serbia and Montenegro.

DNA content of 8 *Iris* species (*I. bosniaca*, *I. germanica*, *I. illyrica*, *I. pallida*, *I. pseudopallida*, *I. pseudopumila*, *I. reichenbachii* and *I. variegata*), was assessed by flow cytometry. The 2C DNA value ranged from 11.48 to 14.01 pg. The significant variations of DNA amount were observed among three populations of *I. bosniaca* (from 11.66 to 12.34 pg), and 4 populations of *I. pseudopallida* (from 12.17 to 14.01 pg), from different geographical origins and ecologically contrasting habitats. Genome organisation and chromosome complements for certain species were characterised by molecular cytogenetic techniques: 1) chromomycin fluorochrome banding for detection of GC rich DNA regions, 2) fluorescence *in situ* hybridization (FISH) with rDNA probes to determine the number and position of rDNA loci, 3) DAPI after FISH experiment revealing number and position of heterochromatic bands and 4) silver staining to study NORs activity. This work presents the first report on genome size and physical mapping of 5S and 18S-5.8S-26S rDNA gene clusters for these species. Some considerations concerning the chromosomal evolution of the endemic Balkan irises are also presented.

P0687. The Cytotaxonomic Studies on the Properties of Species of *Origanum leptocladum* Boiss. and *Origanum micranthum* Vogel

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The flora of Turkey, in terms of medical plants, are too rich. Among these plants, the species of *Origanum* L. which is one of the medicinal plants with high commercial value has occupied an important place. Approximately 70 % of the species of *Origanum* L. are endemic either on an island or in a mountain. When Turkey's flora is examined, it is known that there are 21 *Origanum* L. species which belong to eight sections. 13 of these species are endemic. In this research, the cytotaxonomic properties of two endemic species, *Origanum leptocladum* Boiss and *Origanum micranthum* Vogel which belong to *Origanum* L. genus and show the spread in the East Mediterranean Area of Turkey are examined. The cytotaxonomic properties of these two *Origanum* L. species are first dealt with and by doing their chromosomes counting, they have been given in their original scheme. The numbers of the chromosome of both species have been determined as 2n=30.

P0688. Genetic, morphological, physiological and taxonomic studies in the Chilean endemic genus *Leucocoryne* Lindley

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The genus *Leucocoryne* (*Alliaceae*) is endemic to Chile and is distributed from Iquique (26° 13' S) in the Atacama desert to Concepción (36° S) in the South. It has conservation problems. Genetic, morphological, physiological and taxonomic findings of research in Chile since 1996 are presented. Half of *Leucocoryne* are either 2n=10 or 2n=18. Gene flow occurs between ploidy levels. It is largely self-incompatible. We developed morphological descriptors to characterize 15 species in the germplasm collection. Morphological and molecular (AFLP) variation is found. *L. narcissoides* and as *L. appendiculata*, the two northernmost species, are different at the molecular and flower morphology levels. They are the only ones with six stamens or with fertile staminoids. Seeds store for up to three years at room temperature and germinate at 10-15 °C. The first cycle is completed in approximately 100 days. *Leucocoryne* bulbs break dormancy in 4.5 months at 20°C, but there is variation. Nutrients are remobilized efficiently to the bulb at maturity. Tissue culture and commercial varieties have been developed. *Leucocoryne talinensis* a new species has been described and published.

P0689. Cytological and molecular approach to analyse Patagonian *Bromus* speciesA. M. García¹, G. E. Schrauf², G. González¹, L. Poggio^{3,1}, C. A. Naranjo¹, M. P. Dupal⁴, J. W. Forster⁴, G. Spangenberg⁴;¹CIGEN -CONICET, Llavallol, Argentina, ²Facultad de Agronomía UBA, Buenos Aires, Argentina, ³Facultad de Ciencias Exactas y Naturales UBA, Buenos Aires, Argentina, ⁴Plant Biotechnology Centre, Bundoora, Australia.

Bromus pictus, *B. setifolius* and *B. brevifolius* are taxa of the section *Plnigma*, native from the Patagonia. It is believed that they constitute a specific complex whose morphological trait variation did not permit a clear delineation between the taxa. Variation within this group was detected using AFLP's and RAPD's. *B. brevifolius* has a close affinity with *B. pictus* and both taxa are relatively more distantly related to *B. setifolius*. Cytogenetic analysis revealed that *B. setifolius* is: 2n=28; *B. pictus*: 2n=70; and *B. brevifolius*: 2n=70. The genomic *in situ* hybridization (GISH) of labelled DNA of *B. setifolius* over the 70 mitotic chromosomes of *B. pictus* reveals that the probe hybridized 28 chromosomes, morphologically similar to those of *B. setifolius*. This result confirms that *B. pictus* must be separated from *B. setifolius* and given full specific status. Moreover, GISH confirms the allopolyploid origin of *B. pictus* and the relationship with its putative progenitor *B. setifolius*.

P0690. The relation of the Scrophulariaceae tribes Buddlejaceae and Teediaceae as inferred from nuclear RPB2-d and chloroplast DNA sequences.

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The phylogeny of Scrophulariaceae as inferred from the nuclear low copy gene RPB2-d and the chloroplast DNA regions trnL/F and ndhF is presented focusing on the relations of the tribes *Buddlejaceae* and *Teediaceae* as well as the taxonomically unplaced genus *Camptoloma*. Two RPB2-d paralogues are present in both diploid (2n=38) and tetraploid *Buddleja* species, as well as some putative pseudogenes. The basic chromosome number of *Buddleja* is X=19, and it has been hypothesized that the origin of the genus is a result of a hybridization event between two lower chromosome numbers, most probably X=7 and X=12, of which *Gomphostigma* (X=7) has been suggested to represent one of the ancestral lineages. *Gomphostigma* is here shown to be nested within the *Buddleja* clade, and hence more likely derived from a *Buddleja* ancestor. Inclusion of the genera *Nicodemia*, *Chilianthus* and *Emorya* into *Buddleja* is supported, and a South African origin of *Buddlejeae* is indicated. *Camptoloma* appears closely related to *Teediaceae*.

P0691. Species-level Phylogeny, Biogeography, and Ecology of *Elegia* (Restionaceae, South Africa)

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Restionaceae are Southern Hemisphere graminoids, most diverse in the Cape Floristic Region (CFR, South Africa) and SW Australia. We inferred the phylogeny of the *Elegia* group, which comprises 60 African species, from a cladistic analysis of sequence data from four chloroplast markers (*trnL-F* region, *atpB-rbcL* spacer, *trnK* intron, *matK*, and *rbcL*) for all 60 species. Based on the cladogram we proposed an expanded circumscription of the genus *Elegia*. The biogeographical pattern in *Elegia* was assessed by applying four recently published methods to delimit areas of endemism, and by contrasting a grid overlay with eco-geographical areas to test the influence of the input data. All methods retrieved with varying resolution the same areas of endemism in the CFR. Our results show that eco-geographical areas should be preferred over a grid overlay in the study of biogeographic patterns. Contrasting the overlap of distribution areas with the ecological distance between all sister-species pairs in *Elegia* showed that all sister-species are separated in eco-geographical space.

P0692. An example of species radiation and convergence on a piece of Pacific Gondwana: New Zealand gnaphalioid Asteraceae

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Most of the 70-80 species of gnaphalioid Asteraceae in New Zealand constitute a single lineage that is entirely endemic except for two species in New Guinea. Members of this lineage show poor phylogenetic signals from ITS sequence data but a remarkable morphological and ecological diversity. This is consistent with results from an increasing number of New Zealand's plant groups. These have led researchers to infer rapid and recent species radiation associated with geological and climatic instability in New Zealand in the late Tertiary and early Quaternary. Curiously striking morphological similarities exist between this endemic gnaphalioid lineage and other Asteraceae lineages both in New Zealand and elsewhere. Such morphological convergences can cause taxonomic confusion if they are misinterpreted as synapomorphies. Currently we are using multilocus fingerprinting and sequencing of anonymous nuclear DNA to gain insight into the complex interrelationships of New Zealand's Gnaphalieae.

P0693. Achene evolution in the genus *Ranunculus* L.

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With over 600 species, *Ranunculus* L. is the largest genus in the Ranunculaceae. Modern taxonomic studies have divided this genus into 7 subgenera and about 26 sections. This has been done primarily based on achene morphology, which has been considered the most important source of characters for any infrageneric classifications. Recent molecular studies using nrITS and *matK* data have shown that only some of these sections are monophyletic. This incongruence between molecular and morphological classifications has been attributed to the parallel evolution of morphological characters including achene types. An aim of this study has been to assess the extent of morphological convergence in *Ranunculus* achene morphology and investigate its relationship to habitat. To do this, numerous anatomical and morphological characters have been studied directly and/or under SEM and LM. The patterns of evolution of key characters and reconstruction of ancestral states have been assessed using McClade on a large scale phylogeny derived from nrITS and *matK* sequences.

P0694. Teaching Botany in Aotearoa: Taking the Phylogenetic Approach

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The flora of New Zealand is rich in biodiversity, exhibiting a unique variety of plants including a high proportion of endemic species and distinctive plant forms. As such, the flora provides great opportunities for students of Botany to gain an extensive knowledge of the different plants groups. Because we at University of Waikato are committed to training future plant systematists, our approach is to draw on the most current phylogenetic framework

when looking at plant diversity, with an emphasis on examples from the New Zealand flora. This is especially important as most textbooks available focus almost exclusively on Northern hemisphere taxa. The Angiosperm phylogeny tree modified from APG II (2003) and Judd and Olmstead (2004) underpins the phylogenetic scaffold we employ to aid students comprehension of how groups are evolved. This poster is one example of how we are integrating examples from the New Zealand flora into the current understanding of plant systematics.

P0695. DNA sequences reveal unexpected complexity in the evolution of the New Zealand edelweiss genus *Leucogenes*

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Nuclear and chloroplast sequences signal complex interactions between lineages in the evolution of New Zealand edelweiss species. Two clearly divergent nuclear ribosomal ITS sequence types are found in the genus. One sequence type occurs exclusively in *Leucogenes grandiceps* but some specimens of this species show extensive heteroplasmy in sequences. The other three species of *Leucogenes* (*L. leontopodium*, *L. neglecta* and *L. tarahaoa*) display ITS sequence types similar to a group of pulvinate *Raoulia* species. In contrast to ITS sequences, *Leucogenes* are united by their chloroplast sequences, except for one geographically isolated population. Preliminary sequencing of a novel nuclear marker provides some support for the monophyly of *Leucogenes*. We propose that introgression or hybrid speciation has affected both chloroplast and nuclear ribosomal DNA sequence distribution in *Leucogenes*. Further, the polyploids *L. neglecta* and *L. tarahaoa* appear to have independent origins, and are linked on one hand by their chloroplast sequences to diploid *L. grandiceps* and on the other hand to *L. leontopodium* by their morphology and ITS sequences.

P0696. Assessing genetic variability of New Zealand sea grass (*Zostera muelleri*, Zosteraceae) at multiple spatial scales

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Both the extent and quality of New Zealand's estuarine sea grasses (*Zostera muelleri*) are rapidly declining and are now the focus of ecological and physiological investigations. To contribute to the conservation efforts of this marine angiosperm we surveyed genetic diversity within eight estuaries using Random Amplified Polymorphic DNA (RAPD) markers. Broad scale sampling covered nearly the complete latitudinal gradient of New Zealand. Intensive sampling was conducted within two isolated estuaries along five 10m transects, located on the East and West coasts of the North Island, to assess genetic variation at progressively finer scales. UPGMA, AMOVA, and PCA analyses together confirm that inter-estuary variation was low, while intra-estuary variation was high, with most individuals having a different RAPD profile. This implies that conservation efforts need to be directed towards conserving populations in as many estuaries as possible to retain a high degree of buffering against environmental change and translocation of specimens need to be harvested/ecosourced locally.

P0697. Consensus networks: A tool for studying hybridisation

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Reticulate evolution is a common theme of plant evolution and species radiation. However, when analysing DNA sequences, plant molecular systematists usually depict species relationships on bifurcating trees. Like evolutionary trees, phylogenetic networks provide a means to visualize species relationships, but unlike trees they can also show reticulate relationships. One problem in using networks, is that molecular markers in plants can be characterised by heteroplasmy, recombination, partial gene conversion and paralogy - this can result in networks that are complex and difficult to interpret.

Consensus networks are a new approach that provide a means for combining the strongest phylogenetic signals from different gene loci without losing information (Holland et al, Mol. Biol. Evol. 2004. 21:1459-1461). They also provide a natural way for distinguishing patterns that arise from phylogenetic error and lineage sorting from patterns that arise through hybridisation. Here we report

observations from empirical and analytical studies that illustrate the usefulness of consensus networks for studying hybridisation and plant species radiation.

P0698. Biogeography of endemic New Zealand *Isoetes*

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Isoetes in New Zealand has been determined as two species, *I. kirkii* A. Braun and *I. alpinus* Kirk, alternatively as three varieties or subspecies, and includes diploid and tetraploid plants. Based on morphology and cytology there remains uncertain delineation and taxonomy of endemic *Isoetes*.

Endemic *Isoetes* form a major plant community in South Island lakes, yet have declined in North Island lakes as populations face dual threats from invasive alien weeds and from deteriorating water quality. Although protection of native biodiversity is a goal of lead conservation agencies, for *Isoetes*, suitable selection criteria are needed to identify those lakes, species and populations to target for conservation effort.

Current research seeks to determine the biogeographic scales of genetic variation for *Isoetes* taxa. We used DNA sequences (ITS, cpDNA) and RAPDs to assess the genetic variation amongst numerous populations. The results from this study support the separation of South Island material from North Island tetraploids, and also distinguish a variety described from one lake (Lake Omapere) from which extant populations have already been lost.

P0699. Relationships of model plants in the phylogenetic neighbourhood of *Arabidopsis*

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The model represented by *Arabidopsis thaliana* (Brassicaceae) and its close relatives is becoming increasingly valuable as the already vast array of genetic and biological resources and information available expands. Defining the phylogenetic neighbourhood of *A. thaliana* is an important complement to these resources. Previous treatments of the Brassicaceae have analysed single or concatenated genes. The Z-closure super network method combines trees from genes that are analysed individually to form a "species network". Sets of taxa for each gene need only be partially overlapping and each gene may be described by a different evolutionary model. We present a Z-closure species phylogeny that combines gene trees reconstructed from seven loci. It includes taxa from close relatives of *A. thaliana* that are currently being studied as model species. The resulting network is largely congruent with previously published phylogenies, but identifies incongruence among some datasets. Possible explanations for this are discussed.

P0700. Phylogeny and adaptive radiation in Australasian genus *Celmisia* (Astereae, Asteraceae)

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New Zealand is regarded as fragments of the Gondwana Continent, but is presently located far from the continent. Some genera include a lot of species, and it shows the characteristics like Oceanic Islands. *Celmisia* belongs to Asteraceae, and is endemic to New Zealand and Australia, consisting of ca. 70 species. In especially New Zealand ca. 60 species are distributed. Most of New Zealand species of *Celmisia* are an alpine plant, but some species are the coastal plant. They vary from 1 cm to 1 m in size, and from a small shrub to herbaceous plant in habit, showing speciation with adaptative radiation. The preliminary analysis using the trnL-F region of the chloroplast DNA showed very little mutation in this genus, suggesting that the speciation have occurred relatively recently. Here, we report the result of phylogenetic analysis using sequences of ITS and ETS regions of nrDNA, having faster rate of molecular evolution. In the obtained phylogenetic tree, two major monophyletic clades have been recognized in *Celmisia*. The Australian species located at distal

position in one of the clade, suggesting that they had migrated from New Zealand to Australia in recent.

P0701. Phylogeny and biogeography of *Craspedia* (Asteraceae : Gnaphalieae) based on ITS, ETS and *psbA-trnH* sequence data

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Craspedia (Asteraceae: Gnaphalieae) is a genus of 23 species found only in Australia and New Zealand. Its species have confusing and continuous character variation and indistinct boundaries, which makes their relationships difficult to elucidate. Phylogenetic analyses of ITS, ETS and *psbA-trnH* non-coding spacers resolved three lineages within *Craspedia*, two in Australia and one in New Zealand. The Australian lineages suggest two species radiations into the alpine areas of south-east Australia. *Craspedia* in Tasmania is polyphyletic, with most species closely related to an Australian mainland species. The New Zealand lineage is sister to one of the two Australian lineages. This is consistent with the hypothesis that New Zealand *Craspedia* is derived from a single dispersal event over the Tasman Sea from south-east Australia. *Craspedia* has arrived on Campbell Island and the Chatham Islands by dispersal events from mainland New Zealand. There is low sequence divergence in New Zealand *Craspedia* compared with that in Australia despite more morphological divergence, suggesting a recent and rapid species radiation.

P0702. Molecular dating in the Orchidaceae: inferences about transcontinental disjunctions and explosive diversification in a species rich group with little fossil information

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Orchidaceae is probably the largest family of flowering plants, and practically absent from the fossil record. It has been traditionally considered of young diversification among the monocots, with several transcontinental disjunctions generally explained by long-distance dispersal events. In this study, we used a large (>200) tree compiled from many phylogenetic studies in the family, and optimized *rbcl* sequences with ML. Several different calibrations and NPRS were applied to obtain chronogram estimates for dating the family and selected internal nodes. The crown node was estimated to the boundaries of the Upper Cretaceous to Paleocene, and an early diversification for the main lineages in the family. However, diversification of epiphytic tribes, which account for over 70% of genera and species in the family appears to have occurred in the Eocene. The most important disjunction events are analysed, and indicate overlooked vicariance events, and a new calibration for future studies is proposed at 34 mya with basis in a Boreotropical pair *Phragmipedium/Paphiopedilum*. Inferences with other gene regions are presented to check similar patterns.

P0703. Dating the Asterid tree: Where do Asteridae fossils go?

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The Asteridae represents about 1/3-1/4 of all angiosperms, it includes around a hundred families in ten orders. However, the fossil record of the clade is not reflective of their existing diversity, especially in the two bigger clades; the lamiids (euasterid I) and the campanulids (euasterid II) where most of the diversity of the group is found (42.91% of all eudicots). Different attempts to date the time of origin of the clades that compose this group have resulted in very discrepant estimates, even for the same node (i.e. 29.3 vs. 112 million years for the Asterales). Even when this difference could be due in part to the use of different methodological tools, a great part of it is most likely due to the fossils used for the initial calibration. Reliability of fossils involves a critical evaluation and placement of the fossils into the phylogeny. Here some of the oldest asterid fossils ever reported are re-evaluated and placed into the asterid phylogeny so a minimum age dating was attempted. Preliminary results indicate that the Asteridae (Cornales, Ericales, Lamiids and Campanulids) are at

least 90 million years old, dating back to the Late Cretaceous (Turonian).

P0704. Diversification of Tofieldiaceae (Alismatales)

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Tofieldiaceae are often considered as one of the most morphologically primitive plant groups among the monocots. First, we estimated the divergence time of Tofieldiaceae based on *matK* and *rbcL* gene sequences. The Tofieldiaceae diverged from the Alismataceae complex, which are sister to the Tofieldiaceae, in the Cretaceous. Apart from the Acoraceae, which are most basal among the monocots, the divergence time for the Tofieldiaceae is one of the oldest ones among the living monocot families. Second, we estimated a relationship of infrafamilial taxa of the Tofieldiaceae and their divergence times based on *trnK* (including *matK*), *trnL*, *trnL-F* and ITS data. The genera of Tofieldiaceae (*Triantha*, *Tofieldia* and two monotypic genera) diverged from one another in the Tertiary. In contrast with the recent rapid radiation of the living *Triantha* species, the divergence of *Tofieldia* species began much earlier. In the genus *Tofieldia*, three clades are formed, a clade of *T. calyculata*, a clade of *T. pusilla*, *T. glabra* and *T. okuboii*, and a clade of *T. coccinea*, *T. nuda*, *T. thibetica* and *T. divergens*. *T. coccinea* was rapidly diversified recently into the several varieties.

P0705. The taxonomic and phylogenetic differentiation of the *Potentilla* L. Genus (Rosaceae)

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One of the most numerous and the most variable genus not only in *Rosaceae* family allready in the Dinaric Alps flora and flora of Europea as well is genus *Potentilla*. The broad range of tolerance and large geographic distribution cause of high level diversity of this genus. A lot of species of this genus are «bad species» and their are numerous hybrids between them.

Except of that apomixys is additional endenogenic factor who cause high intraspeci and infracpecis variability of this genus. Those factors in combination with very variable egzogenic factors contributed very complex taxonomy of this genus. Namely, many species, in reference to taxa, have questionable and debatable taxonomic status. Some from them of Balkans and Dinaric Alps unfairly «deleted» from modern floras such as a Flora Europea. In order to it for a long time we paid particular attention on classical taxonomy and phylogeny of the most variable «group of species» of *Potentilla* genus and give their new taxonomy and phylogeny.

P0706. Vegetation and Plant Species Diversity in North East India

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North East India comprising the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura is a distinct megadiversity hotspot. It is located between 20°N to 29°30'N latitude and 89°46' E to 97°30' E longitude. The total geographical area is 2,55,161 km². The floristic diversity is quite enormous in this area but not explored, quantified and estimated into fuller extent and at the finest scale till date. In this paper the plant diversity in North East India is described and highlighted in terms of the species richness and vegetation diversity. Vegetation of this region embraces 5 types - tropical, sub-tropical, temperate, sub-alpine and alpine vegetation. These are further divided into several sub-groups. Diversity of the plant species embraces certain distinct sub-groups namely mono-species genera, endemic and rare species, large groups, economic and medicinal plants, center of origin, wild relatives of cultivated plants, botanical curiosities and primitive flowering plants. The area is comprises of two biogeographic zones - (a) Eastern Himalayas comprising Arunachal Pradesh, (b) North East India comprising other six states.

P0707. Ludwigia (Onagraceae) in São Paulo State (Southeast Brazil)

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Ludwigia L. occupies a rather position in the family Onagraceae. This genus comprising about 80 species in 23 sections. The most important centre of diversity for the genus is South America. The present project was undertaken to investigate the *Ludwigia* species that occurs in São Paulo State, Southeast Brazil. The results will be include a monograph for Flora Fanerogâmica do Estado de São Paulo and one digital version. The capsular and seed morphology provides very useful taxonomic characters. There are 22 *Ludwigia* species known to São Paulo State. *L. hyssopifolia* is a pantropical weed, all the others species are native. The most frequently species collected is *L. octovalvis*. The fruits of section *Myrtocarpus* are obconic capsules with free and pluriseriate seeds. *L. nervosa*, *L. tomentosa* and a rare species *L. brachyphylla* occupy mainly Brazilian savannas localities. *L. lagunae* has the first citation to São Paulo State.

P0708. Flora Mesoamericana: linking specimens with floristics

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Flora Mesoamericana is a Spanish language, long-term, monographic level floristic treatment of the vascular plants of the southern states of Mexico and the republics of Central America. The project combines the revision of the ca. 18,000 vascular plant species of the region with an extensive programme of field collecting in previously uncollected regions, which continues to yield undescribed species and to extend distributions. Planned as a ten volume series, the Flora is also published on-line as W3FM - the first such large-scale floristic project to be presented in this way. The result of a 'revisionary' approach and targeted field exploration has been that approximately 10% of the species described in each of the 2 volumes published to date have been new to science. Flora Mesoamericana and projects like it are much more than mere compilation efforts. We will explore impediments to the completion of such projects and how current databasing efforts in large herbaria can help to improve information content of multi-authored projects such as Flora Mesoamericana.

P0709. A DNA library of Hawaiian plants for taxonomic and natural products research

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The Hawaiian flora represents a model system for the study of evolution, ecology, and conservation. Approximately 90% of the angiosperm and 77% of the pteridophyte floras are endemic, with colonists having traveled over 3600 km from the nearest continental source. Further, colonists have arrived through long distance dispersal followed by in situ speciation from remote continental sources including Asia, the Americas, Australia, Africa, and boreal regions. The potential for expanding our knowledge of plant biodiversity on a global scale can therefore be enhanced by phylogeographic and systematic studies. Our research over the past 13 years has included collecting plant material for development of a DNA library to be used for such investigations. This library now holds nearly 5000 accessions representing endemic and indigenous species of ferns and angiosperms, many of which are rare and endangered. Accessions are available for use by all researchers interested in such studies.

P0710. Urban floras of the eastern part of Male Polissya (Ukraine)

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Results of the preliminary comparative floristic investigation of model urban floras of the eastern part of Male Polissya (Forest zone, NW Ukraine) are presented, including their ecological and structural analyses.

The urban flora of Ostrog (515 sp., incl. 27.2% aliens), Netishyn (687, incl. 23%), Slavuta (558, incl. 26.6%), and Shepetivka (509, incl. 28.8%) are presented. Species of the alien fraction dominate in all urban floras over apophytes. Herbaceous polycarpics, hemicryptophytes, mesophytes, heliophytes; meadow and forest plants prevail in the ecological spectrum of the urban floras; kenophytes and epocophytes prevail in the alien fraction. Most species (80.4% in Shepetivka, and 76.6% in Netishyn) are concentrated in urban, and suburban zones; 17.4% in Netishyn, and 12.8% in Shepetivka, occur in suburban zones only; and 6.9% in Shepetivka and 5.8% in Ostrog are present only in urban zones. Detailed maps (500x500 m) of species distribution in the Ostrog and Netishyn urban floras are presented.

The town flora of the forest zone differs from the regional flora in higher xerophytisation; in small towns these processes are less evident.

P0711. Key to Taxa of Taiwan Grasses with Characters of Leaf Anatomy by ActKey at www.efloras.org

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ActKey: an on-line interactive key for identification. It is different from the traditional dichotomous key in their multi-access character. Rather than having to answer questions to key couplets and follow a predefined path, the use of ActKey is able to adopt different strategies in identifying an unknown plant. The program is web-based and supports most popular Internet browsers. It allows visitors to use a key at the www.efloras.org website for identifications. Moreover, ActKey allows taxonomist to create a key and publish it online. The www.efloras.org website designed by Hong Song is used to host taxon- and specimen-based information, ActKey is one of its many features.

We demonstrated with constructing an ActKey with character sets of leaf anatomy for identifying Taiwan grasses. Fifteen characters were used in the character set with pop-up windows showing images illustrating the character states. Data of microscopic examination on sections of grass leaf blades of 176 species in 5 subfamilies in Taiwan were included. It will be useful as an aid for identifying Taiwan grasses especially when the floral material was unavailable in the identification process

P0712. The Bromeliaceae of Oaxaca, Mexico: Richness and Distribution

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The current knowledge of the Bromeliad flora of the state of Oaxaca, Mexico is presented. Based on the study of 2334 herbarium specimens corresponding to 1331 collections, and a detailed bibliographic revision we conclude that the Bromeliad flora known up to now for Oaxaca comprises 160 species and 15 genera. The 100% of the Mexican species of genera *Fosterella*, *Greigia*, *Guzmania*, *Hohenbergiopsis*, *Racinaea* and *Vriesea* are represented in the state. 22 species are recorded for the first time for Oaxaca and also there are four new taxa, in process of description, represented in the state. Species level endemism reaches 19.37% (31 spp.). Collections of 219 (of 570) municipalities and 29 (of 30) districts of the state were recorded. Among the vegetation types occurring in Oaxaca, *Quercus* forest is the richest with 57 taxa, followed by tropical deciduous forest and cloud forest with 49 and 46 species, respectively. Bromeliad species representation and distribution in Oaxaca are analyzed in detail and also we provide a comparison with those of states of Chiapas and Guerrero.

P0713. Vascular Flora of the Marquesas Islands

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The Marquesas (French Polynesia) are an isolated group of volcanic hot spot islands in the SE Pacific Ocean. These 12 islands range from 61.3 to 330 sq. km in size, from 360 to 1250 m in elevation, and from 1.3 to 6.3 My in age. Steep and rugged, the Marquesas are comparatively botanically unexplored and under-collected. Humans, feral animals, and alien plants have severely

impacted the lowland and mid-elevation vegetation. The native vascular flora comprises ca. 360 species (45% endemic and 30% pteridophytes). The Vascular Flora of the Marquesas Islands is a collaborative project between the National Tropical Botanical Garden, Smithsonian Institution, and Délégation à la Recherche (French Polynesia). Four collecting expeditions in 2003-2004 yielded c. 5800 herbarium specimens comprising 714 vascular plant species. Nearly 60 new species were discovered during the project. Results will be an Internet-based resource and two volume book form publication. The Smithsonian-hosted website provides access to a database of specimens, images, checklist, island distributions, and literature.

P0714. Cooperation between botanical societies and academic institutions

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Many botanical societies have control of information which is of interest for scientific research. It is often available in electronic form although older data may be recorded in other ways.

In a recent project we have made comparative studies on habitats with presumably high species diversity based on information from botanical societies on the occurrence of *Daphne mezereum*. It usually occurs in semi-open habitats in transitions zones containing species from the surrounding biotopes.

Here, we present the mutually beneficial cooperation that has taken place between the members of the scientific project and members of the botanical societies. The primary benefits for the scientific partner have been the sampling of sites for further studies; site identification; and reliable information about the status of different species in different geographical regions. The botanical societies have benefited from updated information about the occurrence of species on previously studied sites; increased respect and acknowledgement of the societies' efforts leading to higher self esteem; and inspiration for new ideas for new studies.

P0715. The vascular flora of the Douro Valley Region (Northern of Portugal)

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The conservation and monitoring of the vascular flora for the Douro Region (Northern of Portugal) is one of the most dramatic problems, specially if we considered the high number of taxa involved (1271 taxa). One methodology is here proposed with the aim of the management of this important natural resource. The physiognomic type, habitat, general distribution, flower-fructification period and common names are used in the analytical matrix, in order to organize the ecological information for this vegetal biodiversity.

The results obtained are pointing out the necessity to adapt the environmental legislation for each region. In this case, the direct application of the environmental directives is one of the most limitative factors faced to the vegetal conservation. The differential behaviour of the taxa, according to the structure of the vegetation per habitat, will be detected from the analysis of the ecological sensibility of the area.

P0716. Digital databasing of type collections from Czech herbaria

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The purpose of the project started in 2001 is to locate and interpret the type specimens of vascular plants preserved in Czech herbaria, and make them available for further research, which involves a detailed nomenclatural and taxonomic study of the specimens, list the specimens in a database, and document the specimens in digitalized form (a database of digital figures of the specimens) both on Internet and on CD-ROM media. In 2001-2003, the most important collection of the tropical and subtropical flora from herb. PR and PRC were evaluated - Australian plants

described by K. Domin (1882-1953), and types from collection of K. Presl (1794-1852) containing e. g. material of Th. Haenke (1761-1816) from South America and the famous fern gatherings used in Presl's monograph of fern genera. Since 2004, types from European collections of F. W. Schmidt (1764-1796), I. F. Tausch (1793-1848), K. Presl, F. W. Wallroth (1792-1857), F. A. Waldstein (1759-1823), and K. K. Münch-Bellinghausen (1752-1838, herb. BRNM) have been digitised.

P0717. Cataloguing the Plant Diversity of Madagascar

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Madagascar, with its high level of plant species diversity and endemism, and growing threats, is recognised as a global biodiversity hotspot. Madagascar's political will to address its conservation issues is strong, but informed decision-making and priority-setting require reliable data, incl. on the delimitation and distribution of species. Conservation planning has been hampered by the lack of a comprehensive Flora or checklist, and estimates of the total number of species and the level of endemism differ widely. Renewed exploration and taxonomic work in the last 2 decades have led to substantial changes in numerous genera, incl. the description of many new spp., and most other groups are in need of review. The *Catalogue of the Vascular Plants of Madagascar* project is an on-line authoritative source with relevant data on each native species. The project team is reviewing and updating the taxonomy of each group, describing new taxa and making nomenclatural changes as needed.

P0718. Toward a new Flora of Ukraine: the concept, progress, and problems

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Vascular plants of Ukraine were treated in several identification manuals, the *Flora of the Ukrainian SSR* (12 vols, 1936-1965), the *Flora of Eastern Europe* (11 vols, 1974-2004), and other floristic publications. However, no modern treatment of the whole vascular flora of Ukraine is available now. Following the publication of a nomenclatural checklist of Ukrainian vascular plants (Mosyakin & Fedoronchuk, 1999; a new extended edition is approaching its completion), botanists of the Kholodny Institute of Botany (Kiev) initiated preparation of a new flora of vascular plants, which will include identification keys, descriptions, and other relevant data. The new *Flora of Ukraine* will complement the ongoing series *Ecoflora of Ukraine* (started in 2000) and current nationwide efforts in biodiversity conservation. The progress of the project and current problems (such as computerization, fundraising, international cooperation, low attention to taxonomy at the national level) are outlined in the presentation. We have to invite more experts in taxonomy, more regional reviewers and editors, and to pay more attention to education of young plant taxonomists.

P0719. An updated commented checklist of the vascular flora of Minorca (Balearic Islands, Spain)

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As most Mediterranean islands, Minorca has a rich flora with a significant number of endemic taxa. However, so far the only specific floristic work about the island was the *Flórmula de Menorca* (1904) of J.J. Rodríguez Femenias, still the most comprehensive study. An updated commented checklist has been completed to commemorate the first centenary of Rodríguez's main work. This new checklist is not only a list of taxa, but also includes additional information on biogeography, life forms, rareness or vernacular names.

A simple quantitative analysis of the previous data shows an overview of some main features of the vascular flora of the island. This latest report establishes a checklist of 1313 taxa. From this total number, the authors have checked the current presence of 1231. The endemic component is represented by 83 taxa (6.3 %) while the alien flora consists of 161 taxa (12.3 %). As could be expected, the Mediterranean component is the most relevant in the biogeographic spectrum (62.9%). In the same way, the life forms reveal the relative importance of the annuals (44.3%). The analysis

of the rareness revealed that up to 30 % of the taxa are very rare (< 5 localities known).

P0720. The pteridophytes of the state of Aguascalientes, México.

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Within the project of "Flora del Estado de Aguascalientes, Mexico", the ferns and fern allies were studied. Fifty-five localities distributed in all municipios of the state were surveyed. Seventy-eight taxa were found: seventy-one species of ferns, five species of *Selaginella*, one species of *Equisetum*, and one species of *Isoetes*. Forty-one taxa are new records for Aguascalientes. Among the ferns, *Cheilanthes* and *Polypodium* are the most diverse and abundant genera, with 14 and 6 taxa, respectively, usually occurring as epipetric in xerophytic associations, subtropical areas or dry forests. *Anemia*, *Anogramma* and *Botrychium* have the most restricted distribution with only few populations in the state, the two formers are located in subtropical scrub, and the last one in open high elevations in *Quercus* forest. *Selaginella* is also common in Aguascalientes, and inhabits rocky cliffs and rocky soils in dry environments. *Equisetum* and *Isoetes* are restricted to subaquatic and marshy places.

P0721. The pteridophytic diversity in Yunnan, China

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This paper deals with the diversity of the pteridophytes of Yunnan, China. Yunnan ranks first among China in its number of pteridophytic taxa. There are 60 families, 198 genera and 1500 species of pteridophytes making up 95.2 percent, 85.7 percent and 57.7 percent of the Chinese totals respectively according to Ching's system. There are also many taxa unique to Yunnan/China, *Phlegmariurus yunnanensis*, *Archangiopteris bipinnata* Ching, *Angiopteris esculenta* Ching, *Angiopteris sparsisora* Ching and *Gymnosphaera austro-yunnanensis* (S. G. Lu) S. G. Lu et C. X. Li are examples. While *Christensenia assamica* (Griff.) Ching and *Platyserium wallichii* Hook. belong to families are only occurrence in Yunnan around China. Yunnan has the richest taxa of pteridophytes in China and this area is one of the richest taxa of pteridophytes in the world. The richest taxa of pteridophytes in Yunnan is a combination of long geological history, complicated geographical environment and sustained biotic evolution. Yunnan's pteridoflora vary from tropical to temperate, in the area exist multiple climate types, it is actually an epitome of Eurasia continent's pteridoflora.

P0722. Importance of conservation of wetland flora: Present scenario of West Bengal State (India)

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Abstract:

Wetlands are among the most important natural resources on earth. In 1990, an inventory of the wetlands in India was carried out by the Ministry of Environment and Forests, recorded ca. 4.1 mha covered by wetlands. In West Bengal, 476 wetlands of different kinds each exceeding an area of 10 ha have been identified. Of these, macrophytic diversity of 18 large wetlands have been explored for the first time by the present authors. Shoreline morphology, water regime, pH, salinity of water and perceived threats were studied. Altogether 201 species of wetland angiosperms were collected. The best macrophytic growth is noted in neutral to slightly alkaline pH (7.50 - 8.31). Floristic analysis, distribution, frequency, phenology, habit and habitats, and the importance of macrophytic diversity for waterfowl habitats and their conservation were discussed. *Aldrovanda vesiculosa* L. is an almost extinct species in this state. Conservation measures must be initiated in making the wise use of wetlands.

P0723. Flora of Altinbesik Cavern National Park (Ibradi-Akseki/Antalya-Turkey)

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In this study, the flora of Altinbesik Cavern National Park were displayed. 1481 plant samples were collected as regards the study

between June 2002 and September 2004. As a result of the evaluation of the plant samples collected, 330 genera, 562 species and 578 taxons belonging to 83 different families were identified. 11 taxons are new for the C3 square. Out of the plant samples diagnosed 9 taxons belong to Pteridophyta and 569 taxons belong to Spermatophyta division. 6 Gymnospermae and 563 Angiospermae, which belong to the Spermatophyta division, belong to the subdivision of Angiospermae. From the subdivision of Angiospermae, 480 taxons belong to Magnoliopsida and 83 taxons belong to Liliopsida class. 248 of the total taxons in the field are Mediterranean elements, 28 Irano-Turanian elements, 19 are Euro-Siberian elements and 283 are multi-regional or are taxons whose phytogeographical regions are unknown. Besides, in the area of study 67 endemic taxons were found. 50 of these endemic taxons are Eastern Mediterranean elements, 7 are Irano-Turanian elements, 1 is Mediterranean element and 9 are not included any geographical location.

P0724. Towards a Catalogue of the Plants of Colombia

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Colombia, with an estimated 30,000 species of native plants, is the second richest country on earth in terms of plants. A multilateral project is currently underway towards the production of a Catalogue of the Plants of Colombia, a highly needed instrument for research, conservation, and sustainable use of Colombian plants. The Catalogue is compiled at the National Colombian Herbarium, Bogotá, and the Herbarium of the University of Göttingen with support of the Volkswagenfoundation. The work will provide a compendium of all vascular plants, bryophytes, and lichens occurring in the country, with information on habitat, geographical distribution, and conservation status. A first draft of the Catalogue is scheduled to be ready by December 2005. This draft is the basis for specialists to produce family treatments. Preliminary checklists are already available for bryophytes (1,600 spp.), lichens (1,500 spp.), and the largest families of vascular plants (10,848 spp.). These figures represent 45% of the number of species expected in Colombia. We plan to complete the Catalogue by December 2008.

P0725. Some Endemic Plants from Southwestern Turkey

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In this study, twenty endemic taxa were investigated. These taxa were *Consolida raveyi* (Boiss.) Schröd., *Papaver spicatum* Boiss. var. *spicatum*, *Hesperis pisidica* Huber-Morath, *Minuartia pestalozzae* (Boiss.) Bornm., *Lathyrus belinensis* N.Maxted & D.J.Goyder, *Ebenus boissieri* Barbey, *Liquidambar orientalis* Miller var. *integriloba* Fiori, *Cephalaria scoparia* Contandr. & Quézel, *Anthemis rosae* Sm. subsp. *carnea* (Boiss.) Grierson, *Centaurea pestalozzae* Boiss., *Erica bocquetii* (Pesmen) P.F.Stevens, *Cyclamen trochopteranum* O.Schwarz, *Convolvulus galaticus* Rostan ex Choisy, *Onosma strigosissimum* Boiss., *Verbascum nudatum* Murb. var. *nudatum*, *Salvia caespitosa* Montbret & Aucher ex Bentham, *Fritillaria whittallii* Baker, *Colchicum baytopiorum* C.D.Brickell, *Crocus baytopiorum* Mathew, *Ophrys lycia* Renz & Taub. Distribution, habitat and ecology, flowering time, altitude and threatened categories of these plants were given.

P0726. Some New Introduced Endemic Taxa from South Anatolia in the Last Decade

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In this study, some new introduced endemic taxa has been investigated from South Anatolia in the last decade. These taxa are *Silene subbuliana* I.G. Deniz & O.D. Düsen, *Astragalus inanae* R.S. Göktürk, O.D. Düsen & Sümbül, *Glycyrrhiza flavescens* Boiss. subsp. *antalyensis* Sümbül, Ö. Tufan, O.D. Düsen & R.S. Göktürk, *Potentilla nerimaniae* H Duman, *Tordylium ketenoglui* H. Duman & A. Duran, *Cephalaria ekimiana* R.S. Göktürk & Sümbül, *Helichrysum orbicularifolium* Sümbül, R.S. Göktürk & O.D. Düsen, *Campanula yaltirikii* H. Duman, *Ornithogalum pamphylicum* O.D. Düsen & Sümbül, *Ornithogalum isauricum* O.D. Düsen & Sümbül,

Allium elmaliense I.G. Deniz & Sümbül, *Colchicum imperatoris-friderici* Siehe ex K.M. Perss., *Colchicum inundatum* K.M. Perss., *Ophrys climacis* Heimeier & Perschke and *Ophrys hygrophila* Gügel, Kreutz, D. & U. Rückbrodt. Description, distribution, habitat, ecology and IUCN 2001 threatened categories of these taxa are given.

P0727. Investigation of flora of National Parks located in Smolensk and Kaluga provinces of west of Russia

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National park "Smolenskoe Poozer'e" (1462 km²) is the unique large protected natural object in Smolensk province. This park is located to the north-west of Smolensk province and includes 35 lakes. National park "Ugra" (986 km²) is the main protected territory of Kaluga province. This park extends from north-west to south-east of the province along the river valleys of Ugra, Zhizdra, and Oka.

We inspected the territories in 1999-2004 and made separate flora lists for multiple landscape units. We also registered biotope for each species. This information was stored as a database and was used for identification of especially valuable territories of the parks. 890 species and hybrides of vascular plants were registered in "Smolenskoe Poozer'e". It represents approximately 72% of flora of Smolensk province (native flora - 80%) 1070 species and hybrides were registered in "Ugra". This number represents 78 % of the flora of Kaluga province (native flora - 80 %). Many species are located closely to its range limits. Only a few of adventive species display wide distribution in natural communities. This investigation was partly supported by RFFI (project 04-04-49641.).

P0728. Historical estimates of biodiversity and the development of identification keys

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The number of species of angiosperms estimated to exist has been fairly stable for 50 years and has been of the same order of magnitude as the number of described species throughout this time. But in the 17th to 19th centuries, the number of described plants and the number of plants estimated to exist were increasing exponentially, from around 6000 known plants in the 1660s to more than 200 000 by 1900. Also, until around 1800, the estimated numbers of plant species were almost always an order of magnitude more than those of described plants. Early botanists faced an information overload. They needed tools to identify and describe plants precisely, to communicate that data to others who lacked access to the same specimens, and that could accommodate all plants yet to be discovered. The experimentation with new information management techniques occurring during this period resulted in the development of the "natural method" -- suited to storing information about all plants in relatively uniform groups -- as well as the identification key -- a technique enabling users to find the names of specimens, making it easier to determine whether they were novel or previously described.

P0729. DNA taxonomy of the genus *Galanthus*, species identification and illegal trade

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The genus *Galanthus* L. comprises 19 species and it occurs naturally in Europe, Turkey and in the Caucasus. Snowdrops are popular garden plants and millions of bulbs are sold annually, mainly *G. nivalis*, *G. elwesii* and *G. woronowii*. Most of the bulbs are collected in the wild in Georgia and Turkey. Sometimes other species, such as those for which trade has been banned, are also collected and declared as *G. elwesii*, *G. woronowii* or *G. nivalis*, for which commercial trade is allowed. *Galanthus* species are presently on CITES Appendix II.

The monitoring of trade in *Galanthus* is very difficult, as plants are usually imported as bulbs and only very limited identification can be undertaken at this stage of the life cycle. Before identification of a *Galanthus* species can be undertaken any bulb has to be grown-

on at a nursery facility for naming at a later date (2-18 months): a costly and time consuming exercise. The custom-houses are interested in quick species identification methods for the genus based on species specific DNA PCR-Marker. *G. nivalis*, *G. elwesii* and *G. woronowii* are genetically clearly different, allowing us the construction of species specific primers.

P0730. Geobotanical zoning in Zagora ranges of Dalmatia, SW Dinarides

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Among Dalmatian uplands so far one studied vegetation chiefly on marginal mountain ranges reputed in alpinism (Velebit, Dinara, Biokovo); the interpolated ranges of Zagora were mostly neglected by botanists. This rugged relief of SW Dinaric Alps in Dalmatian inlands includes 6 calcareous ranges, whose phytocenoses and floral diversity is now studied. For Postak 1446m and Promina 1148m the known botanical data were scarce, and on Svilaja 1509m, Kozjak 1206m, Tovarnica 1265m, and Matokit 1314m nothing was published. On their ridges *Fagus* almost predominates there, and in fresh NE slopes are mesotermic belts of *Quercus frainetto* and *Qu. dalechampii* as in near Bosnia. In xeric SW slopes are Submediterranean belts of *Carpinus orientalis* and *Ostrya*, and in SW foot ravines also *Qu. ilex* and *Phillyrea* occur. The most specific vegetation is on their stormy peaks: deciduous scrub at upper ecotone (*Oreoherzogia*, *Arctostaphylos*, *Ribes*, *Berberis*), and apical windswept balds of *Festuca dalmatica* and *Sesleria robusta* culminating by eroded bare rockfields with *Minuartia capillacea*, *Edraianthus* etc. This zoning of Dalmatian ranges is similar as in Greece and southern Italy.

P0731. Numerical analysis of communities of Classes Asplenetia trichomanis and Thlaspietia rotundifolia in Dinaric Alps region

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Work is based on floristic data, which have been reported over fifty years period of time by eminent researchers in the field of phytosociology in former Yugoslavia. Work also includes results of TWINSPLAN, which has been applied to raw data matrix. Analysis of vegetation in rock crevices has been made by investigating of 106 quadrats, which included 31 plant communities with 372 detected plant species. Investigated were six major regions of Dinaric Alps. Analysis of vegetation of scree and rubble has been made by investigating of 51 quadrats, which have included 12 plant communities with 259 detected plant species. Investigated were four regions of Dinaric Alps. Our work proves distinct relic and endemic character of vegetation in rock crevices and on screes in the Dinaric Alps region, which correlates to its specific geological past and present, and it also proves existence of syngenetical linkage of this type of vegetation in the investigated region. Further more, it is highlighted importance of canyon ecoclimate for survival of great number of endemic species and distribution of submediterranean floral elements into the main land.

P0732. In situ physicochemical measurements of the Cretan endemic genus Petromarula evaluate its adaptability to the certain environmental conditions of Crete

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The presence of an endemic plant in an ecosystem strongly depends on its adaptability to the certain environmental conditions of its habitat. The adaptability of the monotypic endemic genus *Petromarula* was studied on the island Crete, by using JIP-test analyses based on fluorescence induction measurements. Photochemical (quantum yield) and non photochemical (photoprotective) processes were estimated from plants grown in habitats under ambient light intensities (10-1900 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The results showed that *P. pinnata* prefers habitats with low light intensities and is under excitation pressure and stress in high light intensities. This contradicts with the certain light conditions prevailing in Crete (~1700 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The possibility to assess such physicochemical results to answer the question whether the

island of Crete has served as refuge rather than an evolutionary laboratory for *Petromarula* is discussed.

P0733. Endemics and rare plants growing on serpentines in the Rhodopes Mts. (Bulgaria)

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The largest serpentine area in Bulgaria is in the Rhodopes Mts. The serpentine bodies are located within the lower montane belt. The flora and vegetation on these terrains show similarities with such areas in other Balkan countries. The high number of endemics indicates the importance of serpentine habitats as centers for floristic differentiation and speciation. The number of the Bulgarian and Balkan plant endemics found on the study area is 38 taxa. In The Red Data Book of Bulgaria 19 plants of category rare are listed. This number is rather low, compared to other serpentine areas on the Balkan peninsula, bearing in mind the low altitude, the relatively uniform climatic conditions and the short biological history of the serpentine areas in Bulgaria. The link between the taxa of the serpentine flora in the Rhodopes Mts. testifies to a refugial character of the processes. Plant speciation on these areas in Bulgaria is relatively young and a specific floristic complex has not yet developed. A conservation strategy has to be developed in order to protect the serpentines as centres of plant biodiversity.

P0734. Trichome types in European Epilobium and their use for determination

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Although *Epilobium* has been thoroughly studied by several taxonomists, information on trichome structure as distinguishing tool is very scarce. With light and scanning electron microscopy we have recognised two basic trichome types in 14 studied European taxa. All have simple unicellular trichomes with gradually tapering apex and glabrous to papillose surface. Presumably glandular trichomes with truncate to rounded apex and glabrous surface are of two types: long erect and short appressed ones. The latter occur on upper surface of very young leaves of some species and on calyx and fruit of *E. collinum* and *E. obscurum*. Glandular activity of that trichomes has been confirmed by biochemical analyses (TLC, HPLC, reaction with Naturstoffreagent A) which revealed presence of 2 important flavonoids in trichomes and on their surface. Lengths of each hair type are statistically significantly different between some species. So only using quantitative and qualitative data of indumentum all 14 taxa can be reliably distinguished, what is important especially if only vegetative parts of plants are available.

P0735. Vegetation diversity at 17 canyon affluents of Kupa, middle Croatia

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The calcareous ranges of Dinaric Alps are rich in numerous abrupt canyons so far studied chiefly in Bosnia and Montenegro; the canyon vegetation in western Dinarides of Croatia was scarcely known. Kupa river confluence with its 17 tributary canyons of middle Croatia is the most complex system of ramified canyons within Balkans; their rocky escarpments are from 120 to 450m tall. After our recent studies on floral diversity and phytocenoses, its western canyons of upper Kupa, Dobra, Susica, Kupcina and tributaries have mostly a similar vegetation as these ones in SE Alps of Slovenia, including *Pinus nigra*, *Ostrya*, *Fraxinus ornus*, *Cerasus mahaleb*, *Taxus*, *Erica* etc. Other southern canyons of Korana, Mreznica, Slunjica, Tounica and near minor ones have more thermophilic vegetation as in Balkan canyons of Bosnia, including *Acer obtusatum*, *Carpinus orientalis*, *Sorbus umbellata*, *Cotinus*, *Colutea*, *Ruscus aculeatus*, *Satureia montana*, *Ceterach*, *Adiantum*, and also 6 local endemics. This vegetation is linked with frequent foehn impact of southern Mediterranean winds predominating in these southern canyons.

P0736. Genome size of desiccation-tolerant Ramonda serbica and R. nathaliae (Gesneriaceae)

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Ramonda serbica and *Ramonda nathaliae* are extremely rare representatives of resurrection plants among Holarctic phanerogamic flora. Both species are endemics of Balkan peninsula and Tertiary relicts. The sample concerns two populations of *R. serbica* (one from Serbia and one from Montenegro) and one population of *R. nathaliae* from Serbia. Nuclear DNA content and GC percentage were assessed by flow cytometry. The 2C DNA values for *R. nathaliae* and *R. serbica* were 2.38 pg and 7.77 pg, respectively. These results were in accordance with different ploidy levels of these species. The base composition for *R. nathaliae* was found to be 42.06% GC. The significant intra-population variation of DNA amount was observed in *R. serbica* from Montenegro. The 2C DNA content ranged from 2C=7.62 pg to 11.31 pg and revealed existence of different ploidy levels between individuals of this population.

P0737. *Sempervivum kopaonikense* Pancic (Subgenus *Jovibarba* (DC. emend Koch) Baker - groupe *heuffelii* - Crassulaceae) - morphological, chorological and ecological characteristics

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Josif Pancic published *Sempervivum kopaonikense* in "Botanische Ergebnisse einer i. J. 1866 unternommene Reise in Serbien", in Oesterreichische Botanische Zeitschrift, Vienne 17: 173 (1867).

This plant was initially misidentified as *Sempervivum soboliferum* Sims, before to be renamed by Pancic as a new taxon. In the recent floristic literature the name *Sempervivum kopaonikense* has been completely suppressed or classified exclusively as a synonym for *Sempervivum heuffelii* Schott & Syn. *Jovibarba heuffelii* (Schott) LöveC, a species with wide distribution, whose area extends from Dinarides towards Eastern Carpatians, including the main part of Balkan peninsula.

Lectotype of *S. kopaonikense* is designated. On the basis of a biometric study, morphological differentiation between population of *S. kopaonikense* from locus classicus ("Kopaonik, Suvo Rudište") and other ecologically and biogeographically different population of *S. heuffelii* from Balkan peninsula will be discuss. Also, taxonomical, chorological and ecological relations will be analyse.

P0738. Taxonomic revision of genus *Cyanus* (Compositae) in Bulgaria

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In the Bulgarian flora, genus *Cyanus* is represented by 15 taxa, predominantly distributed in the middle and high mountain belts. The territory of the Balkan Peninsula (including Bulgaria) has an important role as a center of origin and diversity of the genus *Cyanus*. It is the richest of endemic species in the Bulgarian flora - more than the half of the taxa have Bulgarian or Balkan distribution. A critical taxonomic revision of all *Cyanus* representatives in Bulgaria with emphasis on their overall morphology, pollen grains, karyotype, ecology as well as the distribution area is made. The pollen grains are Montana- and *Cyanus*-types (Wagenitz, 1955). Four basic chromosome numbers, x = 8, 10, 11 and 12, are detected. The main evolutionary mechanisms in the group are discussed. An identification key is included. The conservation status of the taxa with restricted distribution, according to IUCN criteria, is determined.

P0739. *Polypodium interjectum* and *P. x mantoniae* (Polypodiaceae: Pteridophyta), new to the Bulgarian flora

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Three species of *P. vulgare* complex are known to occur in Europe. These are the diploid *P. cambricum*, the tetraploid *P. vulgare* s. str., and the hexaploid *P. interjectum*. Only *P. vulgare* has been recognized in the Bulgarian botanical literature up to now.

We are reporting one species and one hybrid of *Polypodium* as new to the Bulgarian flora. These are *P. interjectum* Shivas and *P. x mantoniae* Rothm. The latter is a natural hybrid between *P. vulgare* L. and *P. interjectum*.

Plants from several sites were subject to morphological, cytological and spore analyses. The mitotic chromosome counts revealed a pentaploid, 2n=185, for the hybrid specimens, and a hexaploid 2n=222 for *P. interjectum*.

A morphological comparison between Bulgarian *P. interjectum* and *P. vulgare* was made. The frond morphology of the hybrid *P. x mantoniae* is intermediate between the two parents, and the spores were abortive, greatly varying in shape and size.

A map of distribution of the taxa is given. *P. interjectum* and *P. x mantoniae* are known to exist in many European countries. These records for the two taxa in Bulgaria extend our knowledge of their distribution.

P0740. Taxonomic relations between *Juniperus oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *macrocarapa* based on morphological characters

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The aim of the present study was a biometrical comparison of *Juniperus oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *macrocarapa* on the basis of morphological features of cones, seeds and needles.

17 populations of *J. oxycedrus* subsp. *oxycedrus* and 3 of *J. oxycedrus* subsp. *macrocarapa* populations were examined on the basis of features of 30 cones and 30 needles of mostly 20-30 specimen each. Results of the discriminating analysis and the cluster analysis showed significant differences between the two taxa examined. The most important morphological characters discriminating between them were the diameter of the cones and width of needles. *J. oxycedrus* subsp. *macrocarapa* has low level of morphological variation, while typical red juniper was more variable and showed significant morphological differences between the west- and east-Mediterranean populations. The results confirm rather specific than subspecific systematic position of compared taxa and probable origin of west- and east-Mediterranean populations of *J. oxycedrus* subsp. *oxycedrus* from two different Pleistocene refugia.

P0741. Endemic species and vegetation with endemic character in the National Park "Sharr Mountain" - Kosovo

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Abstract

Flora of Kosovo has considered number of endemic and rare species. From this aspect in territory of the National Park Sharr Mountain there are species: *Achillea alexandri* - regis, *Alyssum scardicum*, *Bommulleria diecki*, *Crocus scardicus*, *Dianthus scardicus*, *Draba corabensis*, *Lilium albanicum*, *Melanpyrum scardicum*, *Micromeria albanica*, *Narthecium scardicum*, *Pinus peuce*, *Potentilla doerfleri*, *Saxifraga scardica*, *Sedum flexuosum*, *Sesleria corabensis*, *Thymus doerfleri*, *Verbascum scardicum* ect. Phytocenosis with endemic character in territory of the National Park Sharr Mountain are: *Quercetum trojanae dukagjini*, *Betulo - Verrucosae coritniensis*, *Abieti albae koritniensis*, *Piceetum subalpinum*, *Rhododendro ferruginei - Pinetum peucis*, *Achilleo - Pinetum mugo*, *Pinetum mugo serpentinicum*, *Achilleo - Aubrietum gracilis*, *Potentilla doerfleri - Juncetum trifidii*, *Coccineo - Deschampsietum scardici*, *Trifolieto - norici caricetum*, *Achilleo alexandri - regis - Onobrychietum scardici*, *Ranunculetum crenati scardicum*, *Cynancho - Saponarietum intermediae*, *Sedo - Bornmullerietum dieckii*, *Carici - Nartecietum scardici* ect.

P0742. Classification of iris populations inferred by fruit and seed morphology points at interesting diversification patternM. Biljakovi¹, T. Safner², B. Miti³;¹Ru er Bo kovi Institute, Zagreb, Croatia, ²Faculty of Agriculture, Zagreb, Croatia, ³Faculty of Science, Zagreb, Croatia.

The aim of this work was to classify the iris populations into groups based on fruit and seed characters in order to examine their geographical structuring and speciation processes indicated by previous research.

The material was collected on 16 sites along the Croatian coast, of which 11 associated to *I. illyrica* Tomm. and 5 to *I. pseudopallida* Trinajstić. Eight seed and 4 fruit characters were chosen for their diagnostic value.

MANOVA showed that examined characters could differentiate the species as currently described. Classification (clustering and discriminant analysis) placed populations into 2 distinct zones: the northern part of *I. illyrica* areal (north coast) and the southern part of *I. pseudopallida* areal (south coast), while the central transient zone is represented by populations from neighbouring regions of both areals. Correlation between two types of distances showed that the distance between plants based on morphological characters is to the most extent function of their geographical distance, regardless of the species affiliation.

The results show the need for further study of evolutionary processes and relationships of irises in Croatia.

P0743. Taxonomic relations between *Juniperus thurifera*, *J. excelsa* and *J. foetidissima* based on morphological charactersA. Boratynski^{1,2}, K. Marcysiak², A. Romo³, J. M. Montserrat³, Y. Didukh⁴, K. Boratynska¹, M. Mazur², P. Kosinski¹;¹Polish Academy of Sciences, Kornik, Poland, ²Pedagogic University, Department of Botany, Bydgoszcz, Poland, ³Botanical Institute of Barcelona, Barcelona, Spain, ⁴National Academy of Ukraine, Institute of Botany, Kyiv, Ukraine.

The aim of the present study was a biometrical comparison of *Juniperus excelsa*, *J. thurifera* and *J. foetidissima* on the basis of morphological features of cones, seeds and shoots. *Juniperus excelsa* and *J. thurifera* are considered to originate from the same ancestor, their divergence could take place at the end of the Tertiary, with the climate cooling, as a result of occurring in geographically isolated refuges: *J. excelsa* in SE Europe and SW Asia and *J. thurifera* in SW Europe and NW Africa. Their present ranges are separated, but the species are morphologically similar and additionally *J. foetidissima*, growing in SE Europe and SW Asia, joins them.

5 populations of *J. excelsa*, 4 of *J. thurifera* populations and 2 of *J. foetidissima* were examined on the basis of features of 10 cones and 10 shoots of 18-36 specimen each. Results of analysis of discriminating and the cluster analysis showed great similarity of *J. excelsa* and *J. thurifera*, while *J. foetidissima* was more distant. The results can confirm the thesis of a common ancestor of *J. excelsa* and *J. thurifera*.

P0744. Northern limits of the area of the genus *Onosma* in Slovakia and in the Czech Republic.P. Mártonfi¹, V. Kolarcik¹, L. Mártonfiová²;¹Institute of Biology and Ecology, PJ Safarik University, Kosice, Slovakia, ²Botanical Garden, PJ Safarik University, Kosice, Slovakia.

The area of the genus *Onosma* in the Central Europe reaches 49° of northern latitude. *Onosma tornense* Jáv. (2n=14S, S - short chromosomes) from the sect. *Stelligera* Schur is a primitive taxon occurring in Slovakia. It is a microendemic with unclear relation to balcan species. Another species are the following: *Onosma visianii* G. C. Clementi (2n=18L, L - long chromosomes), which is the most common species in the territory, *Onosma arenaria* W. et K. (2n=12L+8S) and *Onosma pseudoarenaria* Schur (subsp. *tuberculata* (Kit.)Rauschert, 2n=12L+14S). In the Czech Republic the only species occurs *Onosma arenaria*, which has, however, been missing since 1992. In Slovakia all of the four species occur, the occurrence is bound to southernmost pannonian regions of Slovakia, however both number of localities and number of individuals in populations decreases. All of the species are critically endangered in Slovakia and require consistent protection. Support for this research was provided by the Grant Agency VEGA, Slovakia (no. 2/3041/23 and 1/2354/05).

P0745. Biosystematics in *Crocus vernus* agg.

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Caryology, macroscopic morphologic characters and colour patterns in *C. vernus* agg. result in four groups:

1) Western clade: Tepal segments with dark blotches at the outer bases, often with darker veining; corms have shoots only at the tips: diploid: *C. albiflorus*, *C. purpureus*, *C. siculus* (species status unclear), tetraploid: *C. vernus*

2) Eastern clade: Tepal segments with dark blotches at the outer bases and mostly at the tips; large corms have also young shoots from the base or center of the corm: diploid: *C. heuffelianus*, aneuploid: *C. "Cakor Pass"*, tetraploid-aneuploid: *C. discolor*, amphidiploid: *C. exiguus*

3) Southern clade: Tepal segments with dark blotches at the outer bases, inner segments usually with darker veining, outer segments sometimes with blotches at the tips: *C. "sarplaninae"*

4) Southeastern clade: Tepal segments usually without any dark blotches, rarely with white or dark blotches at the tips: *C. tommasinianus*

P0746. Seed micromorphology in European Orchidinae: taxonomical aspects

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A survey of the literature on European Orchidaceae subtribe Orchidinae, reveals that taxonomic interpretations have been primarily made on the basis of morphological characters. Recent phylogenetic analysis of this subtribe (R.M. Bateman & al. in *Lindleyana* 12: 113-141, 1997, R.M. Bateman & al. in *Bot. J. Linn. Soc.* 142: 1-40, 2003) suggested a rearrangement in the systematic of the European Orchidinae, with remarkable changes in comparison with the ancient taxonomical proposals. These analyses provide a suitable frame for the study we present here on the seed testa and its taxonomic implications.

Morphological and biometric analyses have been undertaken, and SEM images obtained from several European species of the genera *Anacamptis*, *Serapias*, *Ophrys*, *Himantoglossum* (incl. *Barlia*), *Neotinea*, *Orchis* (incl. *Aceras*), *Gymnadenia* (incl. *Nigritella*), *Dactylorhiza* (incl. *Coeloglossum*), *Pseudorchis* and *Platanthera*.

General shape of the seed and sculpturing of the periclinal walls are the principal taxonomic characters between the studied genera. Our results show a strong concordance with the phylogenetic analysis cited above.

P0747. Analysis of Essential Oil Composition Revealed Inter-Specific and Inter-Population Differentiation in Two *Salvia* SpeciesM. Maksimovic¹, D. Vidic¹, M. Milos², M. Solic³, S. Abadzic⁴, S. Siljak-Yakovlev⁵;¹University of Sarajevo, Faculty of Science, Department of Chemistry, Sarajevo, Bosnia and Herzegovina, ²University of Split, Faculty of Chemical Technology, Split, Croatia, ³Institute "Mountain and Sea", Makarska, Croatia, ⁴Zemaljski Muzej, Sarajevo, Bosnia and Herzegovina, ⁵Ecologie, Evolution et Systématique, Université Paris-Sud, Paris, France.

Two *Salvia* species (*S. officinalis* L. and *S. brachyodon* Vandas) belonging to the same section (*Salvia*) were investigated. Material of both species was collected at Mt Sv. Ilija (Peljesac Peninsula, Croatia), where they grow together in the same population. Hydrodistilled volatile oils from the aerial parts of the plants were analysed by GC-MS. Genome size and bases composition were determined by flow cytometry.

Despite the same genome size for *S. brachyodon* and *S. officinalis* (0.95 and 0.97pg) and GC% (38.52 and 38.55 %) respectively, and the same chromosome number (2n=14), these two species showed marked differences both in qualitative and quantitative composition of their essential oils. The main components of *S. officinalis* oil were oxygenated monoterpenes, with alpha- and beta-thujone as the major constituents (57.01% and 14.97%, respectively). In contrast, the chemical composition of essential oil of *S. brachyodon* was characterised by high content of sesquiterpenes with humulene epoxide II, as the major compound (22.94%). No intermediary patterns, which display existence of reproductive isolation between two investigated species, were observed.

P0748. The possible ways of speciation in Papaveraceae family

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The species of *Papaveraceae* family have $X = 6, 7, 9, 10$ and 11 . Till now, does not exist unanimous accepted explanations about the speciation ways in this family.

The *Papaver* genus is the biggest one in the *Papaveraceae* family. It is the genus with species strong differentiated as karyological, morphological, physiological, biochemical and serological traits. In *Papaver* genus there are only 3 fundamental chromosomal numbers ($X = 6, 7$ and 11).

To explain the appearance of $X = 7$ and $X = 11$ ($n = 11, 2n = 22$, characteristic for the *Papaver somniferum* species), we are proposing the following hypothesis.

Individuals of species with $X = 6, n = 6, 2n = 12$, by aneuploidy, have produced $n = 5, n = 6$ and $n = 7$ gametes. By random fusion of gametes have appeared the individuals with $2n = 10, 11, 12, 13$ or 14 chromosomes.

From $2n=11$ individuals, by poliploidy, it was possible to appear $2n=22$ ($n=11$) individuals. On the other side, the $2n=14$ ($n=7$) individuals, being tetrasomics, could easy survive.

By investigations on different populations we pointed out similarities between *Chelidonium majus* and *Papaver somniferum* species and between *Chelidonium majus* and *Papaver bracteatum* species karyotype.

P0749. Phylogeny and classification of Rutaceae based on two noncoding regions of cpDNA.

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A phylogenetic analysis of Rutaceae, based on two noncoding regions of cpDNA (the *rps16* intron and the *trnL-trnF* region) was conducted. This study included representatives of all subfamilies and tribes and of most subtribes (65 species in 59 genera, more than 1/3 of the genera in the family). Consensus trees from both separate and combined analyses are largely congruent and consistent with results found in previous *rbcL* and *atpB* studies. The results confirmed that *Cneorum*, *Ptaeroxylon*, *Spathelia* and *Dictyoloma* form a clade sister to the remaining Rutaceae. None of the Englerian subfamilies with more than one genus (except Aurantioideae) emerged as monophyletic. The traditional subfamilial classification of the family is revised, and only two subfamilies need to be recognized: Rutoideae and Spathelioideae. Aurantioideae, even though monophyletic, was reduced to tribal rank (Aurantieae) and included in Rutoideae, in the light of the position of *Ruta*. The circumscription of tribes and some subtribes within Rutoideae will also need to be reevaluated. Characters of the gynoecium, fruits and seeds are not reliable for the circumscription of subfamilies.(FAPESP)

P0750. New insights into the evolution and speciation pattern of Mediterranean Carlina L. (Asteraceae - Cardueae)

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The genus *Carlina* L. (Asteraceae - Cardueae) is mainly distributed in the Mediterranean region, few species expanding into Northern Europe and Siberia. It contains 28 species with a variety of growth forms ranging from woody shrubs to annuals. We examined a nearly complete phylogeny of *Carlina* using nuclear ETS sequence and chloroplast markers. Our results show for instance, that the shrubby habit of species from Macaronesia and Crete is a derived and independently acquired adaptation to the insular habitat as has been shown for several other woody island endemics. Traditionally, it was presumed to be a primitive character of species growing in relict areas. In addition, we defined and compared the ecological niches of all species using GIS data (global information system) of the actual geographical distribution. By combining this with our phylogeny we were able to test whether the speciation events in *Carlina* were caused by ecological or geographical separation.

P0751. Assessing the Atlantic-Mediterranean disjunction of Armeria pungens with AFLP

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AFLP were used to investigate the disjunct distribution (Iberian-Corso-Sardinian) of a diploid coastal shrub species from sand dunes, *Armeria pungens*. We examined variation in 240 individuals from 23 populations that span its geographical range using two different restriction enzyme combinations (*EcoRI/MseI* and *KpnI/MseI*) and three primer pairs. Three genetically divergent groups that correspond to geographic regions were detected: (i) Mediterranean populations (Corsica and Sardinia), (ii) Atlantic Iberian populations north of S. Vicente Cape (including offshore Cies islands NW Spain), (iii) Atlantic Iberian populations east of S. Vicente Cape. In concordance with previously assessed variation for ITS, cpDNA and morphology, populations from Corsica and Sardinia were genetically closer to Portuguese than to southern Iberian. This pattern suggests that Portuguese populations are the immediate source of plants that colonised Corsica and Sardinia. A highly divergent population from the Gibraltar Strait is likely due to introgressive hybridisation with congeners; an interpretation that is also supported by sequence data.

P0752. Iberian endemisms in the Beira-Duriense mountains (Portugal)

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I have been studying the flora and vegetation of a group of mountains situated S of the river Douro and N of the Mondego. This area can be divided in 4 major groups of mountains: Caramulo; Arada/Freita; Montemuro/Lapa; Penedono/Trancoso; and other minor sierras. The height of this area (2000 km²), ranges from 700 to 1382 m. Granites and schists are the dominant rocks. There are 5 Portuguese endemic taxa: *Anarrhinum longipedicellatum*, *Centaurea herminii* ssp. *lusitana*, *Ceratocarpus claviculata* ssp. *picta*, *Murbeckiella sousae* and *Teucrium salviastrum*. The Iberian endemisms are more than 90 (more than 10 % of the total number of taxa: ca. 900), including *Ranunculus bupleuroides*, *R. nigrescens*, *R. olisiponensis*, *Ortegia hispanica*, *Silene acutifolia*, *S. marizii*, *Ulex micranthus*, *Omphalodes nitida*, *Linaria triornithophora*, *Digitalis thapsi*, *Odontites tenuifolia*, *Galium broteroanum*, *Knautia nevadensis*, *Pteroccephalidium diandrum*, *Hieracium castellanum*, *Hispidella hispanica*, *Narcissus cyclamineus*, *Gagea lusitana*, *Paradisea lusitana*, *Crocus carpetanus*, *Festuca summilusitana*, *Koeleria crassipes*, *Periballia involucreta*, *Trisetaria ovata*, etc.

P0753. Phylo-climatic modelling: Phylogenies, Palaeo-climate and Environmental Preferences

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A new technique is demonstrated for the interpretation of the role played by climate in plant diversity through the optimisation of climate variables on phylogenetic trees. This is achieved by calculating climate preferences for individual species based on established bioclimatic modelling techniques using point distribution data from distributed sources. We demonstrate a new technique for optimising the component parameters on phylogenetic trees. These parameters can be re-combined into bioclimatic models for ancestral nodes throughout the phylogenetic tree. The combination of DNA-sequence data and the fossil record is used to establish time-calibrated phylogenies. Using these chronograms, bioclimatic models can in turn be projected into the relevant palaeo-climate scenario to establish possible areas of palaeo-distribution. This technique is demonstrated using exemplar plant groups from Mediterranean-type winter-wet climate zones.

P0754. Characterisation of olive germplasm from Molise region by microsatellite markers

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In the whole Mediterranean basin a large number of varieties of *Olea europaea* are present. This produce a series of problems concerning the germplasm characterization, management and preservation. In addition there is the problem arising from the existence of homonyms and synonyms. This make difficult the cultivar identification. The single sequence repeats (SSR) are co-dominant markers, showing a large number of polymorphisms per primer set and often multiple alleles in a variety, which can be

highly informative. In the present work 21 olive accessions were used, corresponding to the major part of the autochthon germplasm of Molise region.

The identification of 19 genotypes and characterization of 17 olive cultivars with unique genotype allow us to affirm that SSR technology is an efficient tool for genotyping the olive germplasm collection of Molise region and could be valid to distinguish other accessions which can be introduced into the collection.

P0755. Comparison of glandular and aglandular hairs in some endemic species of *Phlomis* L.

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In this study the glandular and aglandular hairs were examined and classified in the various vegetative and reproductive organs of *Phlomis lycia* D. Don, *Phlomis armeniaca* Wild, *Phlomis capitata* Boiss, *Phlomis sieheana* Rech., *Phlomis nissolii* L. The investigated species are endemic to small areas of Turkey. The plant samples were collected from natural population between 2002-2004. It observed that the investigated species have aglandular and glandular hairs of various shapes, sizes and modes of secretion. Certain types of hairs predominant on different organs of the species. The glandular and aglandular hairs differ in variety and density according to the kinds of species and organs related. These characters about hairs will help to distinguish the species from each other. In addition these features of anatomy that the differences in hair morphology are ancillary observations that serve to increase generally the anatomical knowledge of the genus.

P0756. Morphological, anatomical, palynological and ecological studies on *Gynandris sisyrinchium* (L.) Par I.

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The study is based on morphological, anatomical, palynological and ecological investigations of *Gynandris sisyrinchium* (L.) Parl. of which flowers each lasting only a Few hours. The genera *Gynandris* is represented one taxon, only by *Gynandris sisyrinchium* in Turkey. In anatomical studies which used paraffin methods cross-section the plants parts were examined and demonstrated.

In palynological studies, the pollen grains morphology, the types of the pollens and the measurements in polar and ecvatoral using the Wodehouse methods were determined. This study used it has been determined that the plant prefers clayey-loamy textured soil, poor in calcium carbonate, acitic soil and containing varying amounts of organic matter being very rich in nitrogen. The plant grows on nonsaline soils. The analysis of some elements (N,P,K) was carried out in both above and underground parts of this taxon. In addition the chemical analysis of the soil sample belong to the plant was carried out.

P0757. Systematics of Cistaceae

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The Cistaceae, rock roses, is a flowering plant family of malvacean affinity that comprises approximately 180 species. They are showy or small flowered herbs and shrubs that are particularly abundant in sunny areas of the western Mediterranean region, followed by the southeast coastal plain of the United States. The first phylogenetic hypothesis of the relationships within the family, based on nuclear ITS and chloroplast matK-trnK and trnL-F nucleotide data, is presented. The Old World *Helianthemum* are sister to a clade that comprises two subclades. One contains *Cistus*, *Halimium*, and *Tuberaria*, and the other contains New World *Helianthemum* and *Hudsonia*. *Fumana* represents the first branch of the phylogeny, followed by *Lechea*, which is sister to the remaining Cistaceae. Ovule- and pollen-type, staminodes, stigma ultrastructure, funiculus and embryo morphology, mode of sepal development (calyx initiation), leaf arrangement, a host-parasite association, geographical distribution, chromosome number, and a newly described characteristic of the style were found to be among the most valuable nonmolecular characters to the systematics of the family.

P0758. Phylogeny of *Withania* (Solanaceae)

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Withania consists of about 11 species, six endemic in the Horn of Africa region, three in the Canary Islands, North Africa and Spain, one in southern Asia, and one widespread in the Old World tropics and subtropics. A phylogeny was inferred from a data matrix of cpDNA sequences of 2300 aligned positions, as well as from nuclear ITS-data.

We found evidence for the monophyly of *Withania*. The enigmatic *Melissia begonifolia*, endemic to St Helena, is probably *Withania*'s sistergroup and we found evidence for a clade consisting of *Athenaea* and *Aureliana* as sister of *Withania* and *Melissia*. All species of *Withania* except for the Macaronesian and West Mediterranean *W. aristata* and *W. frutescens* form a clade that have *W. aristata* and/or *W. frutescens* as sister group. *W. coagulans* from southern Asia groups together with *W. riebeckii* from Socotra in the chloroplast phylogeny. This appears to be contradicted by the ITS data, and may indicate an allopolyploid origin of *W. coagulans*. The sister of the widespread and important medicinal plant *W. somnifera* is *W. sphaerocarpa* from the Horn of Africa. Very little variation was found in the chloroplast sequences of the Horn of Africa taxa.

P0759. Preliminary phylogenetic analyses of *Sempervivum* (Crassulaceae) inferred from DNA sequence data

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Sempervivum (Crassulaceae) is a medium size genus comprising 50 species of hardy alpine leaf-succulents. The genus is distributed mainly in mountainous regions of central and southern Europe and the Mediterranean. Phylogenetic studies of Crassulaceae confirm that *Sempervivum* is monophyletic and place the small genus *Jovibarba* (five taxa) as sister to the genus; however, other studies have suggested that *Jovibarba* is actually derived within *Sempervivum*. *Jovibarba* was separated from *Sempervivum* based on the differences in flower morphology; *Jovibarba* species have 6-parted, bell-shaped flowers, whereas species of *Sempervivum* have polymerous flowers. There has not yet been a focused phylogenetic study of *Sempervivum*. We used parsimony analyses of DNA sequence data derived from the nuclear (nrDNA ITS) and chloroplast (trnL-trnF) genomes to reconstruct phylogeny for *Sempervivum*. Our analyses indicate that these data will provide a robust estimate of phylogeny for *Sempervivum*. Furthermore, our data suggest that several widespread species of *Sempervivum* are not monophyletic, suggesting a need for a taxonomic revision of the genus.

P0760. Pollen morphology of various Croatian Endemic Plants

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Recently we began a complex study on pollen morphology of Croatian endemic plants. From about 400 Croatian endemic taxa we chose various species narrow endemic to Croatia and/or surrounding areas: 20 taxa from the Genus *Anthyllis*, *Asperula*, *Corydalis*, *Iris*, *Lilium*, *Limonium* and from families Asteraceae, Brassicaceae, Campanulaceae and Orchidaceae. Some of them are very rare and stenendemic for Croatia, such as: *Asperula staliana* Vis. ssp. *arenaria* Korica, *Asperula staliana* Vis. ssp. *issaea* Korica, *Asperula staliana* Vis. ssp. *staliana*, *Corydalis acaulis* (Wulfen) Pers., *Iris rotschildii* Degen, *Limonium vestitum* (C.E.Salmon) C.E.Salmon subsp. *brusnicense* Trinajstić and *Sibiraea altaiensis* (Laxm.) C. K. Schneid. subsp. *croatica* Degen. The pollen samples of all taxa were obtained from field collections and examined by Scanning Electron Microscope in dry and hydrated state. Descriptions of pollen grains will be presented and commented on. Results will contribute to better knowledge about Croatian endemic plants.

P0761. The biodiversity and geography of Eurasian Crassulaceae

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The Crassulaceae (1) is biggest succulent family of Eurasia. It includes 716 species of 25 genera (or 42% of family). Moreover 450 spp. _ cultivated at Europe. In sum it is more than ½ of family diversity (ca. 1700 spp. of 50 genera). The biggest genera of 1 are Sedum _ 250 spp., Rhodiola_90, Sempervivum 50, Hylolephium_30 &c. There are several endemic genera for Eurasia_Meterostachys, Orostachys, Sinocrassula, Prometheum &c. Species of 1 are distributed practically around of all Eurasia, but nevertheless they are spread very partial. The most amount of species are concentrated in mountain region (not less then 75 %). Exist several centers of diversity of 1. The most of species 1 concentrated at China (13 gen. & 230 spp. _ 130 endemics) and in Tibet-Himalaya area (300 spp. or 40% of all Eurasian 1). 2 center _ in S. & C. Europe_60 spp. of Sedum, all Jovibarba, many of Sempervivum and Umbilicus spp. 3 _ in Asia Minor and Caucasus with endemic genera Chistiophyllum, Prometheum and many endemic spp. of Sedum, Phedimus, Sempervivum &c. Some of regions of Eurasia are represented by a small amount of 1. It can be connected with historic, climate and geographical features of these areas.

P0762. Diversity and conservation of genus *Sonchus* sect. *Pustulati*

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Biodiversity evaluation and conservation programs are directly dependent on the quality of taxonomic data. This value is increased when we are dealing with endemic plants. Based on biological and typological species concepts, we review the taxonomy of the endemic group *Sonchus* sect. *Pustulati* in order to obtain the basic information for the conservation of species. It is found that it comprises three taxa (instead of the four taxa distinguished until now), and we propose a taxonomic status change.

The section distribution is limited to about ten coastal or low altitude rocky walls enclaves from western Mediterranean area. Populations show reduced extensions and mean densities ranging from 21 to 55 individuals/100m². We study the population of *Sonchus pustulatus* Willk. from El Barranco del Caballar (Almería) as an example of population viability. It is compound of about 200 individuals and demographic structure indicates that, in spite of high fruit set, viability mainly relies on the survival of adults. Seed dispersal seems to be the factor limiting colonisation of similar enclaves. The role of small reserves is discussed as main way of preservation for the group.

P0763. *Campanula Waldsteiniana* and *Pyramidalis* lineages: Not more than Neighbours - or Truly Relatives?

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European amph-Adriatic and Western Balkan region is floristically exceptionally rich, among other, comprising at least 85 species and subspecies of *Campanula* genus, without many poorly known lower (incipient) taxa and hybrids. Among the most interesting in the region are endemic indigenous lineages, such as "isophylloid" aggregates *Campanula Waldsteiniana* (*C. waldsteiniana* - *C. tommasiniana*) and *Pyramidalis* (*C. pyramidalis* - *C. versicolor* - *C. secundiflora*). Being neither isophyllous nor heterophyllous, but a little bit of both, the *Waldsteiniana* and *Pyramidalis* relationships to other campanuloids in the region puzzle botanists for a long time. Comparative morphological and ecological data, isoenzymes, restriction-site variation of PCR-amplified cpDNA fragments and ITS1-5.8S-ITS2- sequences of the nuclear ribosomal DNA were used to provide better insights into these fine relationships. The data were analysed using multivariate statistical and cladistical methods: here are presented the preliminary results, showing that two morphologically distant lineages might be truly relatives after all.

P0764. An SEM study of peristomes and spores morphology of *Philonotis* (Bartramiaceae, Musci) from the Iberian Peninsula

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The genus *Philonotis* Brid. is the largest genus of the family Bartramiaceae, having 189 accepted species (Crosby M.R., Magill R.E., Allen B. & He S. 1999. A checklist of the mosses. Missouri Botanical Garden). In the Iberian Peninsula only eight of them appear [*P. caespitosa* Jur., *P. calacarea* (Bruch & Schimp.) Schimp., *P. capillaris* Lindb. ex Hartm., *P. fontana* (Hedw.) Brid., *P. marchica* (Hedw.) Brid., *P. rigida* Brid., *P. seriata* Mitt. and *P. tomentella* Molendo].

There are very few works where the peristomes and spores of the Iberian fructified species of *Philonotis* are described and only drawings and light micrographs have been published (Raeymaekers 1983. *Lindbergia* 9: 29-33; Smith 2004. The moss flora of Britain and Ireland). For this reason a complete description of these structures for the species of *Philonotis* from this area, accompanied with SEM photographs, is provided.

P0765. Molecular phylogeny of Ceroxyleae and Phytelephantoideae (Palmae) based on plastid DNA and two low-copy nuclear genes

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Recent data based on molecular, genus-level phylogenies for the palm family (Arecaceae) point to a sister group relationship between tribe Ceroxyleae, with its four genera *Oraniopsis*, *Ravenea*, *Juania* and *Ceroxylon* disjunctly distributed on the Southern hemisphere, and the morphologically highly distinct, trans-Andean subfamily Phytelephantoideae with its three genera *Phytelephas*, *Ammandra*, and *Aphandra*. The disjunct biogeographic pattern of Ceroxyleae, as well as the apparently rapid morphological evolution leading to the Phytelephantoideae, are two issues of palm natural history that are still unsolved. Here, we present molecular phylogenies of Ceroxyleae and Phytelephantoideae, including a total of 29 ingroup species, based on plastid DNA and two low-copy nuclear genes. Although resolution and support is generally low to moderate inside major genera due to a lack of variation in the data, all taxonomic groups are well-supported as monophyletic. *Ammandra* and *Aphandra* form a clade that is sister to *Phytelephas*. *Ravenea* is a likely sister to the rest of the Ceroxyleae, in which *Oraniopsis* appears basally to *Juania* and *Ceroxylon*.

P0766. Migration history and polyploidization in bipolar plants: the genus *Empetrum* (Crowberries)

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The bipolar genus *Empetrum* is taxonomically complex with 1-18 described taxa. It consists of dioecious, hermaphroditic or polygamous plants at the diploid or tetraploid level. We analyzed samples from most of its range using AFLP, flow cytometric, and morphological data to address the origin of the bipolar disjunction and the evolution of polyploidy and breeding systems, and to contribute to delimitation of taxa. The molecular data suggest that the present bipolar distribution resulted from long-distance dispersal of diploids from western North America to the southern hemisphere. We recognized two distinct lineages based on the molecular and morphological data; the northern circumpolar di- tetraploid *E. nigrum* s. lat. and the southern diploid *E. rubrum* s. lat. There was considerable molecular and morphological variation within these lineages. However, whether these lineages represent two species or two species groups, remains unresolved. Our data suggest that hermaphroditism and tetraploidy have originated repeatedly in the northern hemisphere.

P0767. Assessing the phylogeny of *Solanum* sect. *Petota* using cpDNA and AFLP

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The potato (*Solanum tuberosum* L) is a crop with a large gene pool consisting of more than 200 tuber-bearing species. The group has been studied extensively, mainly using morphological characters. Partly due to a complex evolutionary history phylogeny

reconstruction proved difficult. Only recently, molecular data have been used but only for small parts of the potato gene pool. A complete, comprehensive treatment of the systematic relationships between the species is urgently needed to solve taxonomic problems and to allow scientifically based selection of materials for breeding programs.

In this project we will solve the systematic problems of the *Solanum* sect. *Petota*. Around one thousand gene-bank accessions, representing most tuber-bearing species, have been collected from all over the world. First, a backbone phylogeny of the group is constructed by sequencing chloroplast DNA regions *trnL*F and *psbA/trnH* of all (sub)species available. Next, AFLP is used to get a detailed phylogeny of the branches at the accession level. Results of the analysis will be presented at the meeting.

P0768. Assessing the speciation history of closely related wild tomatoes (*Lycopersicon peruvianum* and *L. chilense*)

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Their recent divergence and the diversity of mating systems make wild tomatoes (clade *Lycopersicon*) an ideal plant speciation model system. We used a multilocus approach to assess the suitability of the Wakeley-Hey isolation model in two outcrossing species. This allopatric speciation model assumes divergence without gene flow. We analyzed DNA sequences of 14 unlinked nuclear loci and found that average levels of silent polymorphism are more than threefold higher in *L. peruvianum* than in *L. chilense*. Although the isolation model cannot be rejected by goodness-of-fit criteria, patterns of intragenic linkage disequilibrium are suggestive of historical gene flow at least in some regions of the genome. In combination with the geographic pattern of postzygotic reproductive isolation, our results indicate that speciation occurred under residual gene flow. Therefore, natural selection is implied as one of the evolutionary forces driving speciation processes in wild tomatoes.

P0769. Phylogeographical relationships of the Chilean vascular flora: tectonic vicariance v/s long distance dispersal

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South-western South America has been divided into several phylogeographic provinces mainly based on bioclimatic and vegetational data. On an attempt to identify real floristic units, I classified the 801 Chilean native genera into 13 classes, later regrouped into 7 floristic elements. The neotropical and pantropical genera reach 33% (together), and the temperate genera (boreal and austral) 27%. The austral-asiatic and endemic genera contribute each 9%. I further analyzed the data via track analysis and identified several basic patterns: South Pacific, North Pacific, Andean, Tropical and Cosmopolitan. The analysis suggests tectonic vicariance as the main factor generating the overall disjunct distribution pattern. But recent results arising from molecular dating of lineage divergences suggest that the importance of oceanic dispersal has been strongly underestimated against the predominance of vicariance. A closer analysis of some key genera (e.g. *Nothofagus* and its parasites) let us discuss the confronting dispersal v/s vicariance hypotheses, and the assumptions in cladistic systematics, vicariance biogeography and molecular dating.

P0770. Phylogenetic relationships of Palaua Cav. (Malvaceae) and the positioning of the enigmatic P. sandemanii (Sandwith) Fryxell

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Palaua comprises about 15 species of annual to perennial herbs or shrubs, including some highly polymorphic taxa, particularly those with deeply dissected leaves (*P. dissecta*, *P. mollendoensis*, *P. pusilla*). The genus is endemic to the lomas formations, the so-called fog oases of the coastal deserts of Peru and N-Chile. The mericarps are arranged spirally or in several superimposed whorls in all species - a character not observed among its closest relatives - except in *P. sandemanii*, in which they are disposed in a circle. Nuclear (ITS1, 5.8S, and ITS2) and plastid (*psbA-trnH* spacer) sequence data were used to reconstruct phylogenies, to

test the monophyly of the genus, and to determine the position of the enigmatic *P. sandemanii*. Phylogenies show poor resolution within *Palaua*. However both, the nuclear as well as the plastid data set, support monophyly of the genus, including also *P. sandemanii*.

P0771. Genetic affinities among populations of South-American conifers inferred by AFLP markers

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South American conifer populations are assumed as remnants of wide distributions prevailing before the Quaternary climatic changes. The present work aimed to establishing degrees of genetic affinity among populations of *Araucaria* and *Podocarpus*, by means of AFLP markers and UPGMA, using Jacquard coefficients. Plants from six *Araucaria* and 26 *Podocarpus* populations were analyzed, generally three plants per population, involving areas of Brazil (most populations), Argentina, Bolivia and Chile. Populations of *A. angustifolia* have close affinities among each other and are genetically distant from *A. araucana*. Populations of *Podocarpus* form two clusters, one grouping *P. brasiliensis*, *P. sellowii* (both in Brazil) and *P. montana* (Chile), and the other *P. lamberti* (Brazil) and *P. parlatorei* (Argentina and Bolivia). The results suggest a) the recognition of two species of *Araucaria* and also two of *Podocarpus*, the latter corresponding to the two mentioned clusters, and b) the most ancient populations of *Araucaria* and *Podocarpus* correspond to southeastern Brazil, wherefrom radiations probably occurred toward other regions of South America.
FAPESP, CNPq

P0772. How the pteridophytic diversity of the South Cone took form

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The South Cone (Chile, Argentina, Paraguay, Uruguay, Southern Bolivia and Brazil) shows high pteridophytic diversity in Frozen Yungas, Southern Brazil and Austral Andes. During their building took roles 3 Gondwanic provinces, Western, Eastern and Southern, with different shares, being Africa a weak player.

A "dry diagonal", producing two opposite sections of rain shadows from the North to the South and the miocene marine transgression should have worked later.

The last floristic interchanges between the 3 areas would have been during the pleistocenian glaciations, by displacement of Atlantic costal line to East and through a "Residual Pleistocenian Seasonal Formation Arc", which could have connected Southern Brazil and Yungas, nearby Santa Cruz de la Sierra, where in its latitude is found the frontier between Frozen and Peruvian-Bolivian Yungas. The hilly migrant routes through the Pampas, played another role in its interchanges.

P0773. Phylogeny and evolution on *Salvia* subgenus *Calosphace* (Lamiaceae): a Brazilian view.

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Salvia L. with 900 species is the largest genus in the Lamiaceae. Most of the South and Central American Species belong in the New World subgenus *Calosphace*. This subgenus is divided into over 100 sections, some of which group unrelated species, often from very disparate geographical regions. A DNA analysis using the plastid and nuclear DNA regions (*trnL-trnF*, *ITS* and *psbA-trnH*) was carried out in order to determine the phylogenetic and geographical relationships within *Salvia* subgenus *Calosphace*, and to identify the placement of the Brazilian species. At least four lineages with different distributions are evident: a pan-American clade (*Uliginosae* Group), a Central American clade, Central America/Andes clade and a largely Brazilian clade. The Brazilian clade is sister to Andean species. Issues relating to systematics and biogeography in the Brazilian lineage and relatives are discussed.

P0774. Biogeographic patterns in the flora of southern South America

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According to recent historical biogeographic studies, South America is a continent of hybrid origin with its southern portion closely related to the southern temperate areas, such as Australia, New Zealand, New Guinea, New Caledonia, and Tasmania (reflecting the existence of an ancient austral biota), and the northern portion closely related to the Old World tropics. An approximation of a definition of southern South America may be obtained by looking at the distribution of many individual taxa of plants and animals. Based on these distributions, southern South America is defined here as the area south 30 degrees south latitude. For some authors, the Andean highlands north of 30 degrees south latitude (for instance Puna and North Andean Paramo) should be also included in this area. The objectives of this presentation are to present a biogeographical sketch of southern South America, and a summary of the knowledge of the phytogeography of this area.

P0775. Biogeography and phylogeny of the sandalwood genus *Santalum* (Santalaceae) based on sequence data and morphology

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Phylogenetic analyses using a combination of morphological and sequence data (ITS, ETS, 3'*trnK* intron and *trnG* intron) from all currently recognized taxa in *Santalum* provide evidence for broad-scale, historical biogeographic patterns in the genus. A well-supported basal grade of taxa from Australia supports an origin of the genus on that continent. Multiple long-distance dispersal events must be postulated to account for the distribution of *Santalum* in India and the Pacific. As previously proposed, the Hawaiian species recently treated in sect. *Hawaiiensia* (*S. ellipticum* and *S. paniculatum*) are evidently more closely related to one another than to the other Hawaiian endemics, *S. freycinetianum* and *S. haleakalae*, recently treated in sect. *Santalum* and appear to have resulted from two independent colonization events to Hawaii. The lineage including *S. freycinetianum* and *S. haleakalae* also includes *S. boninense* from the Bonin Islands and *S. insulare* from French Polynesia, Cooks Islands, Henderson Island and the Marquesas Islands, which indicates that Hawaii may act as a source for dispersal to island groups in the South Pacific and the Western Pacific.

P0776. Comparative phylogeography of eastern North American trees

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Single species phylogeographic studies, while providing an interesting story about the individual species, say very little about the biogeographic history of a region. Comparative studies of multiple, unrelated species with different life history strategies are the key to gaining a more complete record of regional history. The purpose of this research is to trace the biogeographic history of eastern North America, using long-lived tree species as study organisms. The choice to include only trees in this study was based on the fact that many trees in the eastern United States have well-preserved and well-documented fossil pollen records, whereas most herbaceous species do not. The fossil information provides some background data on which many hypotheses have been based. Here we present haplotype networks based on non-coding chloroplast sequence data for North American *Fagus*, *Hamamelis*, *Illicium*, and *Liquidambar* to test alternative hypotheses regarding the timing of the disjunction between the United States and Mexico and potential Pleistocene refugia.

P0777. Biogeography of arctic-subarctic plants during the glacial period: molecular phylogeny of *Tofieldiaceae*

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The climatic deterioration from the late Tertiary to the present made development glacier at middle to high latitude of the Northern Hemisphere. The effect on distribution and evolution of arctic-subarctic plants is one of interesting issues. Because glacier

develops in valleys rather than on ridges of mountains, we can expect that arctic-subarctic plants which grow in ridge (rocky place) of mountain easily survive the glacial period than the plants in valley (wet place) in the same mountain area. We conducted molecular phylogenetic analysis of *Tofieldiaceae* [*Tofieldia* 18 samples from 9 spp. (7-20 spp. known in rocky place of mountains with circumboreal distribution), *Triantha* 8 from 4 (4 in wet place of northern part of NA and Japan), *Isidrogalvia* 3 from 3 (5 in SA), *Harperocallis* 1 from 1 (1 in southeastern NA) and *Pleea* 1 from 1 (1 in southeastern NA)] using plastid *matK*, *trnL* intron and *trnL-F* spacer regions. The molecular phylogenetic analyses indicated that the sequence divergences among *Tofieldia* spp. (max 0.03144, av. 0.02047) are higher than those of *Triantha* spp. (max 0.00566, av. 0.00353). These results are consistent with the scenario mentioned above.

P0778. Molecular phylogenetics and biogeography of subtribe Melittidinae (Lamiaceae)

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The five genera *Physostegia*, *Brazoria*, *Macbridea*, *Warnockia*, and *Synandra* are all endemic to North America. Together with the monotypic Eurasian genus *Melittis* and the East Asian genus *Chelonopsis*, these taxa have been classified as subtribe Melittidinae. Previous morphological studies have failed to uncover synapomorphic characters for this group. Our molecular data show *Melittis*, the type genus of the subtribe, as sister to the greater *Stachys* clade. Thus, the monophyly of subtribe Melittidinae is supported neither by molecular nor morphological data. However, the North American endemics do form a monophyletic group based on molecular data. The molecular relationships among these genera are reflected by morphological and cytological data. The expected close relationship between *Warnockia* and *Brazoria* and their sister relationship to the genus *Physostegia* is confirmed. Most of the North American endemics are restricted to the southeast of the continent. Migrations westwards and northwards are correlated with an increase in chromosome numbers as well as several morphological characters.

P0779. Genetic centers of *Juglans regia* L. origin.

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Our researches deal with the centers of beginnings of *J. regia*. The majority of botanists tend to the idea that the primary center of origin for *J. regia* are Asia Minor, Iran, Afghanistan, Transcaucasia, Middle and Central Asia, and also a belt which stretches towards Tien Shan and the Himalayas. Assumptions of researchers about primary and secondary centers of occurrence of *J. regia* are still disputable in some cases.

M.I. Vavilov considered that the primary genetic center of origin of *J. regia* was the Central Asian center which includes Afghanistan, Northwest India, Tajikistan, Uzbekistan and Western Tien Shan, and also Asian center, which includes internal Asia Minor, Iran and mountainous Turkmenistan.

P.M. Zhukovsky and I.G. Komanich specify the secondary genetic center of origin for *J. regia*, which is Moldova. P.M. Zhukovsky also accepts Southwest Europe as the secondary genetic center of *J. regia*. M.K. Vekhov agrees with him, specifying to Romania, Southern Hungary, Bosnia and Thessaly, where this species is a part of wood plantings and forms pure small groves.

P0780. Systematics, phylogeny and biogeography of *Euphrasia* inferred from morphology and molecular markers.G. L. Gussarova^{1,2}, C. Brochmann¹;¹National Centre for Biosystematics, Natural History Museum,University of Oslo, Oslo, Norway, ²Department of Botany, St.

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The genus *Euphrasia* L. (Scrophulariaceae s.l.) includes perennial and annual green parasitic (hemiparasitic) plants distributed in temperate regions of both hemispheres. It is a taxonomically complex group with frequent interspecific hybridisation, and mainly quantitative traits are used for delimitation of sections and species. In this ongoing study, we integrate morphological and molecular evidence to infer phylogenetic relationships between the Northern Hemisphere species and to produce a revised classification. A preliminary maximum parsimony analysis of 100 species based on morphological characters resulted in a partially resolved

phylogeny, supporting so far only some of the traditional subdivisions of the genus at the section level. We are currently sequencing two non-coding cpDNA regions and one nuclear DNA region (ITS) for a large number of the Northern Hemisphere species as well as some Southern Hemisphere representatives, and the inferred molecular phylogeny will be compared to that based on morphological characters. We also present a recent revision of the taxa occurring in the geographical area of the former USSR.

P0781. Circumboreal-circumarctic phylogeography and taxonomy of bog bilberry (*Vaccinium uliginosum*) inferred from DNA, ploidal levels, and morphology

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We address the effect of the ice ages on the phylogeography and taxonomy of the bird-dispersed *Vaccinium uliginosum* L. complex. Populations from throughout its circumboreal-circumarctic range are analyzed for chloroplast and nuclear DNA, morphology, and ploidal levels. Three main cpDNA lineages are identified, which probably diverged before all major glaciations. These lineages can also be distinguished based on morphology. Polyploidizations have probably occurred at least five times at different times and geographic locations. The impact of the ice ages apparently differed conspicuously between geographic regions: northern survival and extensive postglacial migration in the Arctic, isolation of southern European alpine populations, bottlenecks in previously glaciated boreal and alpine areas, and diversification in Beringia. Beringia was apparently important for the initial divergence and expansion as well as for continuous survival during all ice ages, but played a minor role as a source for later interglacial expansions. We are now examining AFLP and ITS diversity in the species.

P0782. Phylogeography of *Quercus robur* L. in Portugal

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Present genetic structure of many tree species was shaped to a large extent by the last glacial period of the Quaternary and postglacial recolonisation processes, and by human impacts. During the last glacial period, temperate forest species were restricted to small areas (termed refugia) with a milder climate. Fossil pollen data from sites across Europe have been used to reconstruct the location of the refugia of deciduous oak species, and the spread from these into their current ranges. Three areas of southern Europe have been identified as major refugia: Iberian Peninsula, Italian Peninsula and the Balkan Peninsula. The present work aims to identify likely micro-refugia in Portugal. The particular ecogeographical conditions in the Iberian Peninsula, with several east-west range of mountains, have possibly played a major role in the distribution of refugia and in the dynamics of postglacial recolonisation.

A common strategy for molecular screening, based on restriction analysis of three PCR - amplified cpDNA fragments, is being used to study the distribution of diversity within and among *Quercus robur* populations.

P0783. A chloroplast phylogeny for 100 of the 122 genera of Cucurbitaceae reveals the family's spatiotemporal evolution

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Cucurbitaceae (760 spp.) present a challenge for phylogenetic work because of many poorly collected and understood taxa. We present a tree based on four chloroplast loci (5000 bases) that includes 140 spp. from 100 gen., all 11 tribes, and all but one subtribe (following the classification of Jeffrey 2004). The tree confirms the traditional division of Cucurbitaceae into Nandiroboideae (=Zanonioidae; 19 gen.) and Cucurbitoidae (103 gen.). Basal groups occur in Africa, Madagascar, SE Asia, and China, with six New World (NW) groups nested within them. Within Nandiroboideae sits one NW clade (15 spp.), and within

Cucurbitoidae sit four or five NW clades, namely a subclade of Luffeae (3 spp.), the highly distinct Cucurbitaeae (97 spp.; spiny pantoporate pollen) and Sicyeae (126 spp.; polycarpate pollen), and previously unrecognized N American (21 spp.) and S American (52 spp.) subclades of Coniandreae. Eocene to Miocene Laurasian fossils of Cucurbitaceae plus the nesting of NW in OW clades suggest entry of Cucurbitaceae into S America from N America. Most of Jeffrey's tribes are supported by our data, pointing to conserved sets of morphological characters.

P0784. A geographical analysis of the *Caryophyllaceae* family within the flora of Ukraine

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According to the geographical spectrum, the family *Caryophyllaceae* within the flora of Ukraine is represented mostly by Ancient-Mediterranean, Steppe-Eurasian and Holarctic elements. Within the *Caryophyllaceae*, 81 species (37.8%) belong to the Holarctic type of area, and most of them (43 species or 22.0%) belong to the European class. The Steppe-Eurasian type of area is presented by 67 species (31.3%), within which the Pontic group of areas includes 42 species (19.6%), and most of these taxa are comparatively young Steppe ones. The presence of a large number of endemic and subendemic species of the both Steppe-Eurasian (Pontic group) and Ancient-Mediterranean (Crimean group) types testifies to the autochthonous origin of the core of the Pontic and Crimean groups. However, there was a considerable influence of the allochthonous elements on the development of the *Caryophyllaceae* within the flora of Ukraine because of a lot of their species having widely areas.

P0785. A geographical analysis of the genus *Linum* L. in Ukraine

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A geographical analysis of the genus *Linum* L. (*Linaceae*) in the Ukrainian natural flora (21 species) is presented. According to the classification of geographical floristic elements (Kleopov, 1990), Ukrainian *Linum* species are represented by 5 types of geographical elements: Submediterranean (10 sp.), Mediterranean (3), European (2), Nomadic (2), Eurasian (1), and 1 transitional type (3 sp.); and 11 geographical elements: East-Submediterranean (4 sp.), Crimean-Caucasian (3), Crimean (2), East-Mediterranean (2), Pontic (2), European-Mediterranean (2), Central European (2), Carpathian-Balkan-Asia Minor (1), Middle European-Submediterranean (1), Mediterranean (1), and European-Asian (1).

There are two centers of species diversity of the genus in Ukraine: Crimea (15 sp., incl. 2 endemic), and southeastern Ukraine (10, incl. 2 endemic). The diversity of the species composition of *Linum* in Ukraine is determined by ecological, geographical and historical factors of their formation.

P0786. Phylogeny and biogeography of *Cardamine* (*Brassicaceae*), with emphasis on section *Cardaminella*

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The genus *Cardamine* (*Brassicaceae*) is cosmopolitan, but most taxa inhabit boreal, alpine or arctic habitats in the northern hemisphere. The 200-250 species, representing diploids to high polyploids, are divided into 12-15 sections. One section (*Dentaria* L.) has previously been inferred to be polyphyletic based on molecular data, but no whole genus phylogeny is available. Section *Cardaminella* Prantl. is predominantly arctic-alpine and contains about ten species, and has a centre of diversity in Eastern Asia and Western North America. We use nuclear and chloroplast DNA sequences from 80 species of *Cardamine* representing different continents and sections to reconstruct the phylogenetic and biogeographic history of the genus. Of particular interest is to test the monophyly of section *Cardaminella*, and to identify the source area(s) for colonisation of the Arctic for this section.

P0787. A geographical analysis of the Caryophyllaceae family within the flora of Ukraine**M. M. Fedoronchuk;**

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According to the geographical spectrum, the family Caryophyllaceae within the flora of Ukraine is represented mostly by Ancient-Mediterranean, Steppe-Eurasian and Holarctic elements. Within the Caryophyllaceae, 81 species (37.8%) belong to the Holarctic type of area, and most of them (43 species or 22.0%) belong to the European class. The Steppe-Eurasian type of area is presented by 67 species (31.3%), within which the Pontic group of areas includes 42 species (19.6%), and most of these taxa are comparatively young Steppe ones. The presence of a large number of endemic and subendemic species of the both Steppe-Eurasian (Pontic group) and Ancient-Mediterranean (Crimean group) types testifies to the autochthonous origin of the core of the Pontic and Crimean groups. However, there was a considerable influence of the allochthonous elements on the development of the Caryophyllaceae within the flora of Ukraine because of a lot of their species having widely areas.

P0788. Correlations and differences between local and general ranges of vascular plants and mosses**M. Zajac, A. Zajac;**

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Local ranges of chosen vascular plants were accepted after Distribution Atlas of vascular plants in Poland (A. Zajac & M. Zajac eds. 2001), the general ranges after existing atlases of Meusel and Hultén schools and collaborators, but for mosses local and general ranges of Atlas of the Geographical Distribution of Mosses in Poland (R. Ochrya & P. Szmaja eds. 1983-1994) were used. During comparison analyses the universal rules were noticed. The general ranges of mosses are in principle extent and comprise eg. the Holarctic as a whole. Ranges of vascular plants in the most cases are considerably tightened. However local ranges of mosses and vascular plants in the territory of Poland can be classified to the same distributional types. Such a phenomenon is probably connected with the joint history of taxa expansion from the two different groups in period of the late glaciation and the Holocene.

P0789. Phylogeny and biogeography of Balsaminaceae based on chloroplast trnL-F sequences**Y. Song, Y. Yuan, P. K pfer;**

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Balsaminaceae are a morphologically diverse family with two genera, Hydrocera (one species) and Impatiens (~ 1000 species), distributing primarily in the highlands and mountains of the Old World tropics and subtropics area. In order to understand further the phylogeny of this family and clarify the biogeographic relationships among the main diversification centres, we obtained chloroplast trnL-F sequences for 138 samples of Balsaminaceae worldwide and one species of its closest relative Marcgraviaceae. Phylogenetic analyses applying Kimura 2-Parameter distance estimates and Neighbor-Joining method confirmed the monophyly of the genus Impatiens. The topology of the trnL-F tree is in principle congruent to that of our earlier ITS tree, both implying that extant Impatiens species are of Southeast Asian origin, from where dispersals to Southeast Asian islands and North America, to central Asia and Eastern Europe via the Himalayas, and to India and Africa have occurred. The Madagascan Impatiens shows an African origin.

P0790. Taxonomic diversity and diversification of Araliaceae in India**A. K. Pandey¹, J. Wen²;**¹TM Bhagalpur University, Bhagalpur, India, ²The Field Museum, Chicago, IL, United States.

In India Araliaceae are represented by 16 genera mostly in N and NE region and a few in South India. They are: *Aralia* (11 spp.), *Arthropodium* (1 sp.), *Brassaiopsis* (ca. 10 spp.), *Dendropanax* (1 sp.), *Eleutherococcus* (2 spp.), *Gamblea* (1 sp.), *Hedera* (1 sp.), *Heteropanax* (1 sp.), *Macropanax* (3 spp.), *Merrillioanax* (3 spp.), *Panax* (3 spp.), *Polyscias* (2 spp.), *Schefflera* (ca. 30 spp.), *Tetrapanax* (1 sp.), *Trevesia* (1 sp.), and *Tupidanthus* (1 sp.). The

11 species of *Aralia* belong to three sections. In India, sect. *Aralia* has two species: *A. cachemirica* in Kashmir and *A. tibetana* in Sikkim. Indian sect. *Dimorphanthus* has four species with *A. malabarica* endemic to South India. *Aralia* sect. *Pentapanax* has five species with *A. leschenaultii* disjunct between NE and South India. *Panax assamicus* is similar to *P. wangianus* from West Central China, in their narrow leaflets, elongated rhizomes with short internodes, and fruit color. The ITS data suggest that *P. assamicus* is clearly distinct. Two clades can be recognized within Indian *Brassaiopsis*: (1) *B. aculeata*, *B. glomerulata*, *B. hainla*, *B. mitis*, *B. shweliensis*, and (2) *B. hispida*, *B. griffithii*, and *B. simplicifolia*.

P0791. Molecular phylogeny and biogeography of two genera of Northern Hemisphere lamioid mints; *Lamium* L. and *Galeopsis* L. (Lamiaceae)**M. Bendiksby, V. A. Albert;**

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We have examined the phylogeny and biogeography of two Northern hemisphere lamioid genera, *Galeopsis* and *Lamium*, by comparing non-coding chloroplast and nuclear DNA sequences to morphological, cytological and distributional data. The distribution of *Galeopsis* and *Lamium* is temperate Eurasian with centers of diversity in Europe and the Irano-Turanian/Mediterranean region, respectively. An extensive taxon sampling of both genera, including some putative hybrid taxa, was included in this study. Our results indicate that *Galeopsis* is monophyletic, and previously suggested subgroups within *Galeopsis* are supported. *Lamium*, however, is not monophyletic. As currently circumscribed, *Lamium* is paraphyletic with the monotypic genus *Lamiastrum* and the SW-Asian genus *Wiedemannia* as subclades. Furthermore, few of the previously suggested taxonomic subdivisions within *Lamium* are supported. Both genera are prominent in the Fennoscandian flora and appear to have invaded Fennoscandia several times based on our phylogenetic results.

P0792. Species Level Phylogeny and Biogeography of the Dogwood Genus *Cornus* (Cornaceae) Inferred from *matK* and ITS Sequences and AFLP Data**Q. Y. J. Xiang, D. T. Thomas, W. H. Zhang;**

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Cornus is a horticulturally important genus with approximately 55 species divided among 10 subgenera in four major morphological groups. We employed ITS and *matK* sequences and AFLP data to reconstruct a species level phylogeny of the genus with the goal of clarifying relationships among species. Results from phylogenetic analyses support the monophyly of most subgenera with the exception for Subgen. *Cornus* and Subgen. *Kraniopsis*. Subgenus *Afrocrania* and Subgen. *Sinocornus* are nested within Subgen. *Cornus*. Subgen. *Kraniopsis* is monophyletic if *C. peruviana* is excluded. Within Subgen. *Kraniopsis*, species from North American form a clade separated from species from Eurasia. Relationships within each of these clades are not well resolved by the sequence data but better resolved by AFLP data. A new classification scheme better reflecting the phylogeny will be proposed. Phylogenetic dating employing penalized likelihood and Bayesian methods and dispersal-vicariance analysis including fossil species suggested five intercontinental dispersals between Eurasia and North America via the North Atlantic land bridge from the earliest Tertiary to the Miocene.

P0793. Biogeographic diversification of Altingiaceae in the Northern Hemisphere.**S. M. Ickert-Bond, J. Wen;**

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The Altingiaceae are a well-defined group of ca. 15 species that show a classic Asian/ North American disjunct intercontinental biogeographic distribution. To resolve species-level relationships and biogeographic diversification we have generated nuclear sequence data from the *GBSSI* gene, chloroplast data from the *trnL-trnF* intergenic spacer, the *rps16* intron, the *trnS-G* intergenic spacer, and the mitochondrial *nad1-bc* intron. Parsimony and Bayesian analyses resulted in congruent relationships among several lineages. Most notably, (1) *Liquidambar* is paraphyletic; (2) *L. acalycina* and *Altingia gracilipes* constitute a separate Asian tropical to subtropical lineage; (3) *Semiliquidambar* nests within the *L. formosana* lineage; and (4) the eastern North American *L. styraciflua* groups with the western Asian *L. orientalis*, but is highly

distinct from other lineages. Our morphological cladistic analysis supports the monophyly of *Liquidambar* with *Altingia* as its sister. The discordance between the morphological and the molecular data sets may be due to different rates of morphological evolution as well as convergence in these taxa.

P0794. Floristic changes on the Kem-Ludy islands (Russian White Sea, 1962-2004)

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The lists of flora of Kem-Ludy archipelago (Russian White Sea, near Arctic Circle) produced in years 1962 and 2001-2004 were investigated by different methods of data analysis. The full species list contains 284 species. 27 islands were studied, four new islands were appeared due to the tectonic events. Main dynamic features of islands and island group's floras and of species areas were clarified. Most important factors are island square and openness (accessibility for winds). The close vicinity with species-rich island does not prevent the species disappearance. The presence of forest, ecotops formed by crowberry (*Empetrum* spp.) and *Sphagnum*-bogs are most stabilizing factors whereas riches of rocks play negative role in the floristic stability. Marine birds are most important biological factor for island flora maintenance. Many of effects depend on shoaling of channels between islands and probably on improvement of local climate.

P0795. Origin of the genus *Allium*: place and time.

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The genus *Allium* is currently regarded as a member of the Alliaceae, subfamily *Allioideae*. *Allium* is distributed almost exclusively in the northern hemisphere and 88 % of the 780 species are distributed in Eurasia.

Genus *Allium* is subdivided into 13 subgenera. Most of the subgenera show one center of diversity respectively while subgenus *Amerallium* is distributed in all three continents i.e., North America, Europe and Asia. Current molecular studies (ITS and cpDNA) support the monophyletic origin of the genus *Allium* and it is subdivided into two major clades i.e., subgenera *Nectaroscordum* (x=9), *Amerallium* (x=7) along with monotypic section *Microscordum* (x=8) versus remaining subgenera of genus *Allium* (all x=8). The phylogenetic position of subgenus *Amerallium* as sister to Mediterranean subgenus *Nectaroscordum* and east Asian section *Microscordum* provides evidence for an Old World (x=7,8,9,10) origin of the genus *Allium*.

One conspicuous feature of the ITS data is the unusually large intrageneric genetic distances within *Allium*. These molecular differences together with the distribution area of *Allium* indicate an origin of the genus early in the Tertiary.

P0796. Spatial structure of a willow hybrid complex in European rivers

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The tree forming willow species, *Salix alba* L. (white willow), *Salix fragilis* L. (crack willow) and their hybrid *Salix x rubens* are widely distributed tetraploids across Europe in riparian zones and wetlands. 2000 individual willow trees distributed along major rivers or their tributaries across Europe were analysed with codominant introns of consensus gene families and with dominant ISSRs. The co-dominant markers were ideal markers to identify these *Salix* species, their hybrid and the introgressed hybrids. Much of the ISSR variation is kept within populations of *Salix alba*. Significant relationships between the individual *S. alba* phenotypes based on ISSR markers and their position along a N-S and a W-E gradient in the Western plains and Central Highlands ecoregions were found. For *S. fragilis* a significant relationship was found along a W-E gradient in the Western and Central Highlands ecoregions. Introns and ISSRs could give useful information at different geographical levels.

P0797. The pteridophytic diversity in Yunnan, China

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This paper deals with the diversity of the pteridophytes of Yunnan, China. Yunnan ranks first among China in its number of pteridophytic taxa. There are 60 families, 198 genera and 1500 species of pteridophytes making up 95.2 percent, 85.7 percent and 57.7 percent of the Chinese totals respectively according to Ching's system. There are also many taxa unique to Yunnan/China, *Phlegmariurus yunnanensis*, *Archangiopteris bipinnata* Ching, *Angiopteris esculenta* Ching, *Angiopteris sparsisora* Ching and *Gymnosphaera austro-yunnanensis* (S. G. Lu) S. G. Lu et C. X. Li are examples. While *Christensenia assamica* (Griff.) Ching and *Platyserium wallichii* Hook. belong to families are only occurrence in Yunnan around China. Yunnan has the richest taxa of pteridophytes in China and this area is one of the richest taxa of pteridophytes in the world. The richest taxa of pteridophytes in Yunnan is a combination of long geological history, complicated geographical environment and sustained biotic evolution. Yunnan's pteridoflora vary from tropical to temperate, in the area exist multiple climate types, it is actually an epitome of Eurasia continent's pteridoflora.

P0798. The chloroplast PCR primer database: tools for comprehensive phylogeographic analysis of a whole genome.

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A data base is presented which collects published PCR primer information for chloroplast DNA (<http://bfw.ac.at/200/1859.html>). Chloroplast genes evolve slowly, and many PCR primers have been designed to work across species ('universal primers', Taberlet et al. 1991). Amplicons are either the genes themselves, typically in studies of sequence variation in higher-order phylogeny, or spacers, introns, and intergenic regions in studies of phylogeographic patterns within and among species. The current list of 'generic' primers consists of more than 350 sequences. Our experience shows that many of these primers can be combined into pairs quite freely if 'generic' PCR conditions ('two-step PCR') are applied. With this set of primers it becomes possible to study the whole chloroplast genome for variation in a comprehensive way for many taxa. Efficient methods for analysing polymorphisms are necessary - traditional sequencing may not be an option in large-scale studies. Alternatives are simple PCR-RFLP in gels, or denaturing high-performance liquid chromatography for simultaneous detection and analysis of polymorphisms. Examples will be given.

P0799. Molecular defined migration pathways of rare species of the genera *Lathyrus* and *Oxytropis* (Fabaceae)

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Studies of a large nrDNA data set of the cloned and sequenced ITS region and 5' ETS revealed a detailed insight into intraspecific and intraindividual genetic variability of the morphologically and genetically conserved *Oxytropis pilosa* in contrast to *Lathyrus pannonicus* originally sharing the same Eurasian steppe habitat. The evaluation of the molecular data allowed a defined reconstruction of inter- and postglacial migration patterns. During the westward migration of populations of *L. pannonicus* obviously an ecological shift from xerothermic to wet habitats occurred - inferred by phytosociologic classifications. Numerous mutations characteristic for populations within the disjunct relict habitats could be detected; they are linked to habitat preference and support the assumption that the ecological shift is the driving force for biodiversity. The presently unclear classification of some *L. pannonicus* subspecies due to diffuse morphologic characters and ecologically undefined or intermediate habitat use can now be explained by interpreting such populations as relicts of a transition zone and as a result of repeatedly endured (re-) colonisation.

P0800. Phylogeny and phylogeography of myrmecophytic Macaranga species from the Malaysian region

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Twenty-nine of the about 300 species of the palaeotropical tree genus *Macaranga* (Euphorbiaceae) are obligate ant-plants, or myrmecophytes. We used ITS sequencing and AFLP profiling to

reconstruct a phylogeny of 37 species from sections Pachystemon, Pruinosa, and Winkleriana, the only sections containing myrmecophytes. Whereas the resolution achieved by the ITS data set is limited, the AFLP trees support the monophyly of sect. Pruinosa and sect. Pachystemon, and also suggest a subdivision of sect. Pachystemon into four well-defined groups. The same set of taxa was screened for polymorphisms within non-coding chloroplast DNA. More than 50 haplotypes were obtained, and subjected to a phylogeographic analysis. Among closely related species, the distribution of haplotypes correlates with geographically circumscribed regions rather than with taxonomic boundaries, suggesting either chloroplast introgression or ancient lineage sorting. Nuclear microsatellites are currently being developed to complement the phylogenetic and phylogeographic data sets by classical population genetic studies on a local scale.

P0801. Phylogenetic relationships and population structure of species of *Hypochaeris* sect. *Seriola* (Asteraceae, Cichorieae) in the western Mediterranean Basin based on AFLP analysis

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The genus *Hypochaeris* L. includes c. 15 species in the Mediterranean Basin. Two of its four sections, sect. *Seriola* and sect. *Hypochaeris*, form a monophyletic group. Sect. *Seriola* ($2n = 12$) is the sister group of sect. *Hypochaeris* ($2n = 8, 10$). Sect. *Seriola* is composed of four species: *Hypochaeris leontodontoides* (endemic to the Middle Atlas, Morocco), *H. rutea* (endemic to the Betic Cordillera, Spain), *H. achyrophorus* (Circummediterranean) and *H. laevigata* = *H. alliatae* (Central and East of the Mediterranean Basin and North West of Africa). Except for *H. achyrophorus*, which is annual, all the other species are perennials. From ITS sequences we know that *H. leontodontoides* is sister to *H. achyrophorus*, *H. rutea* and *H. laevigata*; and *H. achyrophorus* is sister to *H. rutea* and *H. laevigata*. Amplified fragment length polymorphism (AFLP) has been used to resolve relationships among species and populations. These relationships, plus other factors such as geographical distribution, ecology, breeding system, karyotype, and geological history of regions, are used to formulate hypotheses about factors leading to speciation in this group.

P0802. Phylogeography of multiple polyploid lineages in the rare slim-leaf onion (*Allium amplexans*).

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Polyploidy is an important evolutionary mechanism in flowering plants. Within species, ploidy differences occur both within and among populations, and can occur in closely related lineages, suggesting that polyploid evolution is an ongoing process rather than a rare, macroevolutionary event. The western North American slim-leaf onion (*Allium amplexans*) is found from southwestern British Columbia to southern California. In this species, diploid, triploid and tetraploid plants ($2n = 14, 21, 28$) occur in both mixed and homogeneous populations. DNA sequences in the TrnT-L chloroplast region differ among populations in at least 24 indels and 4 base substitutions. These differences imply two phylogenetically distinct lineages each containing diploids and polyploids, suggesting that polyploids have arisen independently at least twice in this species. Chloroplast haplotype diversity is highest in the Sacramento Valley of California while a small subset of haplotypes occurs in British Columbia, Washington and northern Oregon, where populations are largely triploid and asexual.

P0803. Predicting plant species distributions using altitude and climatic parameters: the case study of African *Ancistrocladus* Wall. (Ancistrocladaceae)

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We test the methods proposed by Skov and Borchsenius (1997) for African species of *Ancistrocladus* Wall. (Ancistrocladaceae), using elevation, mean annual temperature, and annual precipitation variables combined with the existing locality records for each species. We predict the potential and probable distributions of taxa and generate species richness maps of both potential and probable distributions for all taxa. Using these distributions, we assess the collection effort of the genus at a

mesoscale pattern of ecosystem properties: i.e., how well do the existing collections coincide with the species distribution and richness models?

In general, species with a large number of records produced good models. The Gulf of Guinea arc and Gabon, and Sierra Leone to Liberia in Upper Guinea, along with very isolated points in the Congo basin of the Democratic Republic of Congo, are the areas with a high ratio of potential to probable species richness, indicating the areas that are potentially poorly collected, i.e., not well explored. Supplemental fieldwork is needed to find additional areas for the collection and conservation of poorly represented taxa.

P0804. Chloroplast and nuclear phylogeography of two peat mosses in Europe

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Phylogeographic structure of *S. palustre* and *S. fimbriatum* has been investigated in Europe using chloroplast (*trnL-L-F*, *rpl16*) and newly developed nuclear (GapC gene) markers. Resolution power of the *rpl16* gene was higher than that of the *trnL-L-F* region. The single copy GapC gene resolved twice as much haplotypes as the chloroplast genes and it seems to be applicable in population level studies.

Both chloroplast and nuclear genes supported the existence of three main strongly differentiated nested clades in Europe: Central-European, North-European and a South-European clade. Surprisingly the European clades were more divergent than the North-American and European ones, indicating recent intercontinental dispersal. High divergence among the European clades implicates long lasting past isolation and recolonization at least from three different refugia following the LGM. In the case of *Sphagnum palustre*, chloroplast markers separated a mountain and a lowland clade, which might be also in relation to recolonization or specialization processes. To obtain higher resolution, development of SNP markers as well as AFLP and ISSR analyses are in progress.

P0805. Phylogeographical observations on species complex *Ophrys fuciflora* inferred from nuclear ribosomal ITS sequences

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In order to clarify the phylogenetic relationship among species from *Ophrys fuciflora* complex nr ITS region of 19 taxa, representing 44 populations from 13 European and North-African countries, was sequenced. We found that the nrITS types were strongly correlated to geographical regions, while they were totally shared by the species in a discrete area. The geographical pattern of the nrITS types is assumed to relate to the primary glacial refugia in Southern-Europe where the different nrITS types had been separated during last glaciation. Surprisingly overlapping sequences were detected north of the Mediterranean peninsulas in the Carpathian-basin, South-Germany and Eastern-France, which could be separated as different paralogous sequences after cloning the PCR products. The paralogues imply recent hybridization in the abovementioned geographical regions, where the expanding postglacial lineages may have met forming hybrid zones. Since paralogous sequences thought to have rapid merging into the domineering ITS type, the hybrid zones seem to be recent ones on postglacial time-scale.

P0806. Maternal and paternal DNA lineages reveal geographic genetic structuring among the populations of *Rumex acetosa* (Polygonaceae)

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The weed *Rumex acetosa* is one of the few angiosperm species which have sex chromosomes (XX in females and XY₁Y₂ in males). The two Y chromosomes are assumed to be of a common origin, and they are believed to recombine during meiosis. Such a homology brings about individual male plants that may be either homozygous or heterozygous in regard to those areas. The presence of Y chromosomes allows investigations of paternal DNA lineages, while analyses of chloroplast DNA allows examinations

of maternal DNA. In this study, DNA sequence polymorphisms present in a 850 bp Y-chromosomal section, representing heterochromatin, and in a 409 bp chloroplast section (trnL-trnF spacer) of *R. acetosa* originating from several European locations were investigated. In the chloroplast area only one substitution and two deletions resulting in four haplotypes were detected while in the Y-chromosomal DNA 179 substitution sites and four deletions/insertions were discovered. Concerning 73% of the substitution sites, heterozygous individuals were found among the sampled males. The pattern of sequence variation detected *R. acetosa* revealed considerable geographic structuring.

P0807. Phylogeography of pantropical sea-drift seed dispersal plants III -- Speciation through sea-drift species: *Canavalia rosea*

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The genus *Canavalia* Adans consists of ca. 50 species mostly distributed in tropics and sub-tropics of the world. Four subgenera are known in the genus: subgen. *Cantodonia* and *Wenderothia* are distributed in the New World, *Maunaloa* is endemic to Hawaii, and *Canavalia* has pantropical distribution because of the presence of the pantropical sea-drift seed dispersal plants, *C. rosea*, in the subgenus. To clarify if the present distribution pattern of the genus was caused by the seed dispersal of *C. rosea*, we performed molecular phylogenetic analysis using extensive samples. Phylogenetic tree constructed by ITS sequences of nrDNA showed a clear clade consisting of members of subgen. *Canavalia* and *Maunaloa*. All the Hawaiian endemic species, as well as, some species that have sea-drift seeds were in the same clade together with numbers of samples of *C. rosea* obtained from vast range of its distribution. This result suggests that the Hawaiian endemic subgen. *Maunaloa* was derived from *C. rosea* that migrated to Hawaii by sea-drift dispersal, and loss of sea-drift ability occurred in the speciation process.

P0808. Phylogeography of pantropical sea-drift seed dispersal plants IV -- Classification and phylogenetic relationships of *Entada phaseoloides* (Leguminosae; Mimosoideae) and its allies.

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Entada phaseoloides is famous for its gigantic sea-drift seeds, which are sometimes washed ashore in coastal regions distantly apart from its original habitat. *E. koshunensis* is an endemic species to Taiwan and very similar to *E. phaseoloides*, and the systematic treatments of these species have been confused. In order to reveal their relationships in detail, we performed molecular and morphological analyses using extensive samples obtained from the Ryukyus, Taiwan, Vietnam, Samoa, Vanuatu, and Australia including samples obtained from the type locality of *E. koshunensis* in Taiwan. Molecular phylogenetic tree using about 3,700bp sequences of cpDNA was clearly separated into two clades. One is composed of samples from Taiwan, the Ryukyus and south Pacific, for which small convex seeds are common characters. The other clade is composed of samples from Vietnam, Taiwan, and two islands of southern Japan, for which large compressed seeds are common. The difference of the two is conspicuous, and the former will be corresponding to *E. phaseoloides*.

P0809. Vascular plants of Ukraine as perfect models for phylogeography

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The rich and diverse vascular flora of Ukraine contains many relict, endemic and otherwise interesting taxa, which are often subjects of taxonomic, phytogeographical, and paleobotanical controversies. Many of these controversies can be efficiently solved by phylogeographical methods. Phylogeography is currently a booming field, especially in Europe, but Ukrainian plants were

barely touched by such studies. The author attempted to identify the most interesting and promising groups of Ukrainian plants suitable for phylogeographical studies in cooperation with European botanists: endemic and relict species, taxa with disjunctive ranges, some trees (e.g., taxonomically complicated *Betula* spp., model taxa of *Quercus*), and other plants crucial for understanding the historical patterns of Ukraine's flora (and the European flora in general) in the Pleistocene and Holocene. Geographically such taxa are concentrated in the Carpathians, Crimea, the Volhynian and Podolian area, and some parts of the steppe and forest zones. Phylogeographical data should be correlated with extensive data of paleopalynological studies accumulated in Ukraine.

P0810. Phylogeography of pantropical sea-drift seed dispersal plants I -- Scope in General and a case of *Ipomoea pes-caprae* (L.) R. Br. (Convolvulaceae)

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"Pantropical Sea-drift Seed Dispersal Plants" have extremely wide distribution area that spread over the littoral areas of tropics of the five continents. This intriguing distribution pattern is thought to be the results of long distance seed dispersal of their sea-drift seeds. To clarify how this unique distribution pattern has achieved, to estimate the levels of migration among populations to maintain the unity of the species over the world, and to reveal the speciation process through these species, extensive field and molecular works have been done for four representative plants: *Ipomoea pes-caprae*, *Hibiscus tiliaceus*, *Entada phaseoloides*, and *Canavalia rosea*. In this presentation, we introduce a general scope of the study of pantropical sea-drift seed dispersal plants, and a case study on *Ipomoea pes-caprae*. CpDNA analysis showed no nucleotide substitutions over 4kb length among samples of *I. pes-caprae*. On the other hand, considerable amount of genetic variation in an intron of nuclear DFR-B gene. A phylogenetic tree of DFR-B gene of nrDNA suggested clear geographic differentiations.

P0811. Molecular phylogeography of *Castanopsis* forests in Japan, based on mtDNA variation of weevil insects

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In order to elucidate the past distribution and colonization routes of broad-leaved evergreen forests in Japan, we have compared the intraspecific phylogeographical patterns of chloroplast DNA (cpDNA) among 6 plant species growing in *Castanopsis*-dominant forests. However, the molecular evolutionary rate of plant cpDNA is relatively slow at the nucleotide sequence level. In this study, we tried to use intraspecific variation in mitochondrial DNA (mtDNA) of phytophagous insects for the phylogeographic study of host plants. The mtDNA of weevil *Curculio* proved to have a greater amount of intraspecific variation than that of the cpDNA of the plants distributed in the same geographical region. The geographical boundary between the two groups of mtDNA haplotypes found in *C. hilgendorfi* lies in the Chugoku-Shikoku region. This pattern could be consistent with the result based on the intraspecific phylogeographical patterns of cpDNA variation among 6 plant species growing in *Castanopsis*-dominant forests, which suggested the existence of additional important refugia from Muroto to the Kii Peninsula along the Pacific coast of Japan during the glacial ages.

P0812. Postglacial chloroplast phylogeography of the autotetraploid *Biscutella laevigata* L. (Brassicaceae) in the Western Alps

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Biscutella laevigata is a perennial herbaceous species supposed to have colonized the Alps before the Würm as a diploid (2x) and have thereafter recolonized this area as an autotetraploid (4x). This study points out the putative 2x parent populations of the Western Alps 4x by counting chromosomes and analysing new polymorphic markers on chloroplast DNA in 60 populations.

Thirty haplotypes were analysed by Median-Joining network to get insights into the evolutionary history of this young polyploid complex.

Most of the 2x populations show well differentiated, rare but fixed, haplotypes and are interpreted as interglacial depauperate relicts. Tetraploids display haplotypes belonging to different lineages located in the northern and the southern parts of the western Alps. The southern 4x are linked to different Italian 2x haplotypes. The northern 4x populations share their haplotypes with a relictual 2x population located in a peripheral refugia and interpreted as the origin of this lineage.

Post-glacial recolonisation of *B. laevigata* is characterised by polytopic autopolyploidy out of refugia and admixture of expending lineages in the central Alps.

P0813. Population history of the *Euphrasia transmorrisonensis* (Orobanchaceae) complex in Taiwan based on isozyme data

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Genetic distribution pattern of populations of *Euphrasia transmorrisonensis* complex in Taiwan has been recently studied. Nested clade analysis based on internal transcribed spacer region of nuclear ribosomal DNA and chloroplast DNA sequences has shown a strong association between genetic data and geographical location. In this study, isozyme data, which served as a non-neutral genetic data, were used for further investigation. Three well resolving polymorphic loci, AAT-1, MDH-5, and SkDH-1, including nine alleles were detected and combined to 14 haplotypes. These haplotypes were reconstructed to a nested cladogram using a majority consensus tree of the 82 most parsimonious ones. The associations of haplotypes and geographical location were then analyzed using GeoDis 2.0 with homozygote individuals (192/ 676 individuals) in 23 populations. The contingency test indicates that haplotypes and geographical location are associated. The restricted gene flow with isolation-by-distance can also be inferred for the population history at the lower clade level.

P0814. Phylogeography of pantropical sea-drift seed dispersal plants II – Massive introgression in *Hibiscus tiliaceus* and *H. pernambucensis* (Malvaceae)

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To understand how genetic differentiation leading to speciation proceeds in pan-tropic sea-drift seed dispersal plants, we focus on *Hibiscus tiliaceus* and its allied species. *Hibiscus tiliaceus* is one of sea-drift seed dispersal plants widely distributed in the tropics of the Old World. One of its allied species, *H. pernambucensis*, is only in the New World, and the total distribution ranges of the two spread over the earth in tropics. Three other related species, *H. elatus*, *H. glaber*, and *H. hamabo* are distributed in restricted areas. We performed sequencing analyses, PCR-SSCP and PCR-SSP analyses using cpDNA in more than 1000 individuals around the earth. The molecular data demonstrated that *H. tiliaceus* could be the source of speciation for all the allied species, and they had frequent migration by sea-drift seeds among the populations. Furthermore there is obvious genetic subdivision between populations of *H. tiliaceus* and *H. pernambucensis* over the east Pacific Ocean, but in the Atlantic, there is some semblance of genetic homogeneity contrary to their distribution boundary. This might suggested cytoplasmic introgression occurred between the two species.

P0815. Phylogeography of *Hymenaea stigonocarpa* (Leguminosae-Caesalpinioideae): a tropical tree from the Brazilian Cerrado.

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The polymorphism level and spatial distribution of chloroplast genome variation were investigated in populations of *Hymenaea stigonocarpa* (Leguminosae). *H. stigonocarpa* is a neotropical tree that occurs mainly in the Cerrado (a savanna vegetation), a Brazilian biome classified as hotspot for conservation priorities.

Twenty-six different haplotypes of the chloroplast noncoding region between the genes *psbC* and *trnS* were identified in seven populations (13° - 21°S and 41° - 48°W). Mean haplotypic diversity within populations, total haplotypic diversity and differentiation among populations (s_T) were 0.70, 0.91 and 0.46, respectively. Phylogeographic analyses showed a clear association between the haplotype phylogeny and geographic distribution of populations, revealing distinct groups probably related with vicariance events. A hierarchical analysis of molecular variance corroborated this hypothesis, indicating that most of the variation can be attributed to differences among these groups (57%) and only 5% to differences among populations within groups.

P0816. Discriminant analysis of isozymic data on classification of Brazilian species of *Cryptocarya* (Lauraceae)

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This study evaluated isozymic data in combination with morphological criteria for classifying Brazilian species of *Cryptocarya*. Through the analysis of 41 polymorphic isozyme loci, 124 alleles were recorded from 739 adult trees of 35 natural populations of ten species of *Cryptocarya* native to the Atlantic rain forest. Isozyme loci that best discriminated among pre-defined populations of different species were selected by stepwise discriminant analysis. Using a *k*-nearest-neighbour algorithm, the classification of individuals into their respective populations resulted in 92.8% correct classification. Similarly, the quadratic discriminant function generated from the selected loci that best discriminated among pre-defined species, using 2-nearest-neighbours, resulted in a resubstitution of individuals accounting for 94.15% correct classification. Results demonstrated that discriminant analysis of isozymic data can be used for efficient marker-based allocation of individual trees into pre-defined groups of populations and species of *Cryptocarya*, complementing and/or confirming information obtained from classical taxonomic studies.

P0817. Band pattern or allele frequencies in phylogeographic analysis of AFLP data: How much does it matter?

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Amplified Fragment Length Polymorphism (AFLP) is a universal and increasingly used method to investigate population structure and phylogeography, especially in genetically little known organisms like many plant species. A drawback of AFLP's is the dominant nature of the data, which prevents a straightforward estimation of allele frequencies. Two approaches are common: statistical analysis is either based on similarities among individual band patterns, or allele frequencies are estimated assuming Hardy-Weinberg equilibrium. The first avoids assumptions which are difficult to check for many plant populations, but the second allows a more powerful and sophisticated data analysis. Here I compare these two approaches in inferring the phylogeography of two arctic-alpine ampho-atlantic flowering plants, whose main distribution area is in Europe: *Arabis alpina* and *Saxifraga stellaris*. The recolonization history of the formerly glaciated regions in northern Europe is inferred and migration rates within and among regions are addressed.

P0818. Mountain flora of Sila (S Italy). Statistics, ecological characteristics and phytoecography

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Mountain ecosystems in Mediterranean region, representing "islands" of wild Nature, are object of different scientific studies aiming to describe the patterns of floristic diversity and suggest causes of this pattern; to develop methods for predicting how biodiversity may change; and to discuss the consequences on the ecosystem. Sila Mt., localized in S Italy, represents the northernmost part of Calabrian arc, a siliceous Ercinic complex including S Calabrian and NE Sicilian mountains. Data about mountain floristic complex of Sila, situated is presented. Like Mountain flora is considered the species occurring in beech (*Fagus sylvatica*) and Calabrian pine (*Pinus nigra ssp. calabrica*) vegetation belt reaching altitudinal range (800) 1000 - 2000 (1927) m. For every species are identified: attitude to basic rock, attitude to water regime, life form (after Raunkiaer), vertical distribution

(valuated using altitudinal steps of 500 m) and horizontal distribution (geoelement). For the analysis the concept of "concrete" flora is applied and some hypothesis for flora relations and consolidation are evaluated.

P0819. The spatial patterns of the flora in large European cities

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For more than 25 years, in many European cities, researchers conduct intensive mapping of spontaneously occurring species of vascular plants. Such research was conducted in several capital cities: Berlin, Brussels, London, Rome, Warsaw, Vienna and Zurich, and also in other German (e.g. Bielefeld, Bochum, Duisburg, Münster) and Polish cities (Poznań, Krakow, Lodz). The research has enormous significance from the documentary and practical, as well as cognitive, point of view. On the basis of the analysis of several thousands of range maps, it may be concluded that in the impact zones of large urban centers, specific ranges of plant species and the unique spatial structure of the flora are formed. They present an essential relationship with the forms and intensity of the land use, and in particular, with the structure of built-up areas. Those factors have a considerable impact, among others, on the spatial layout of the species abundance of the flora and the layout of species of a varied geographical-historical origin and various ecologic requirements. Those relationships comprise the special patterns of the flora, presented on the poster.

P0820. The Caryophyllaceae Family in Argentina: an Atlas.

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Caryophyllaceae is present in Argentina with 23 genera and 119 species. The indigenous genera are: *Cardionema* DC. (with 4 species), *Colobanthus* Bartl. (3), *Drymaria* Willd. ex Roem. et Schult. (5), *Philippiella* Speg. (1), *Polycarpea* Lam. (1), *Pycnophyllopsis* Skottsb. (1) and *Pycnophyllum* Remy (7). Genera introduced present only with 1 species are: *Agrostemma* L., *Dianthus* L., *Holosteum* L., *Petrorhagia* (Ser.) Link, *Saponaria* L., *Scleranthus* L. and *Vaccaria* Medik. Genera present with indigenous and introduced species are: *Arenaria* L. (8), *Cerastium* L. (14), *Herniaria* L. (3), *Paronychia* Mill. (7), *Polycarpon* L. (4), *Sagina* L. (4), *Silene* L. (25), *Spergula* L. (12) and *Stellaria* L. (14). The first part of the Atlas contains a description of each genera, a key of differentiation, a short description of each species and a map with its distribution in Argentina. The second part is bound to illustrations of each species including habit, flower parts (sepals, petals, stamens, gynæceum and fruit), and photographs from seeds and pollen grains obtained with scanning electron microscope.

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P0821. Geographic patterns of tree taxa in the Brazilian Atlantic Rain Forest

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To investigate how arboreal taxa are distributed in the Brazilian Atlantic Ombrophilous Dense Forest domain we selected 63 surveys and analyzed their occurrence on neighboring phytogeographic formations ("cerrado", seasonal semideciduous forest, and montane forest). After checking synonyms, we came to 1,498 valid binomials in 465 genera and 104 families. We defined seven possible geographical patterns, and analyzed the presence of each taxon in each survey at the levels of species, genus, and family. No species occurred in more than 33 surveys, and species, genera and families showed a quite similar geographic distribution. No taxon showed disjoint distribution, and most species and genera showed a Southeastern-restricted geographical pattern, thus indicating that the Southeastern coast could act as a diversity center for the whole Atlantic Ombrophilous Dense Forest. Analysing the occurrence of taxa in neighboring formations, we corroborate the concept that the Atlantic Forest (Mata Atlântica) is a very large phytogeographic province south of the Amazon and east of the Andes, which includes forest and non-forest formations.

P0822. Floristic Relationships of Seasonal Forests in Northeastern Brazil

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Northeastern Brazilian seasonal forests were examined to determine if they contain one or more floristic groups and possible correlations between the spatial distribution of abiotic factors and the forest communities were investigated. A TWINSPLAN analysis comparing the distribution of genera present in different sites located between the regional climatic extremes - the semi-arid *caatinga* and the moist Atlantic Coastal Forest separated the areas in two groups: Group 1 with up to 8 dry months per year and/or a total annual rainfall >1000 mm, with floristic links with the Atlantic Forests, and Group 2 with more than 8 dry months per year and/or a total annual rainfall <1000 mm. Group 2 is further divided into two clades, one corresponding to the semi-arid core, within the *caatinga* domain, and the other occupying the eroded peaks as well as the western slopes of the Borborema Plateau, where the driest seasonal forests are found. Ours results suggest that the Borborema Plateau functions as a geographic barrier separating the humid and dry floras, and that 1000 mm/year is the upper limit for the present distribution of dry plant formations in northeast Brazil.

P0823. Irano-Turanian endemic plant species of SE Iran

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South-east Iran with high plateau and solitary mountains was considered as a part of Irano-Turanian region. The area has an important situation in case of phytobiodiversity. The vegetation of the area is mostly consist of stepic plants and somewhere with scattered shrubs. Although some Irano-Turanian species in this area are common to west Pakistan, Afghanistan and central Iran flora, some of them are endemic. Almost 1/4 of endemic plant species are specific of SE Iran. Among these plants, only 6 species belong to *Liliopsida*. *Asteraceae* with 2.9 percent and *Fabaceae* with 1.8 percent have the most species. Some of other families which have endemic species are *Plumbaginaceae*, *Lamiaceae*, *Apiaceae*, *Rosaceae* and *Caryophyllaceae*. Most of endemic species which are specific of the area belong to genera *Astragalus*, *Cousinia*, *Acantholimon*, *Nepeta*, *Silene* and *Ferula*. It is noteworthy that most of species of genera *Cousinia* (9 species) and *Acantholimon* (7 species) are specific of this area.

P0824. Relicts of former cultivations in Central Europe

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The flora of each area contains species that are relicts of cultures conducted ages ago. Nowadays, many of them are not used any more, and they are not even connected with arable plants. The group of such species is commonly called the relicts of cultivations. Both foreign (the so-called ergasiolypophytes) and native (the so-called eciolipophytes) arable species for a given flora may be included in this group. Some relicts of cultivations occur only on the sites of former cultures, others - have widely spread in favorable conditions.

The relicts of cultivations may be classified on the basis of the historic periods when they were cultivated, e.g. the relicts of pre-historical or medieval cultivations. Moreover, the names of some groups of relict plants derive from the name of tribes that had brought and cultivated them, e.g. plants from the Slavic times, or from the name of structures, next to them they survived, e.g. "earthworks" plants.

Relicts include former healing plants (among others, *Malva alcea*), former dyeing plants (*Isatis tinctoria*), decorative species (e.g. *Viola odorata*), spices (among others, *Allium scorodoprasum*).

P0825. Characterization and efficiency of the floristic inventory methods employed in the Madidi National Park, Bolivia

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We compare effort [number of persons/day × number of days] and efficiency [number of species/effort] in an inventory of the woody plant species in a quantitative floristic inventory of 152 plots of 0.1 ha (all stems >2.5 cm dbh) in 14 sites and 8 plots of 1 ha (all stems ≥ 10 cm dbh) in seven sites in the Madidi National Park, Bolivia. We inventoried mainly tropical rainforests below 1000 m, but also dry and montane forests. As expected, with the plots of 0.1 ha we observed a lower effort (5±1.4) and a higher floristic efficiency (18±3.3) than for the 1 ha plots (25±9.1; 5±1.8). In general, the plots both of 0.1 and 1 ha located below 1000 m elevation have a lower number of stems and a higher number of species than plots above 1000 m. Within the 0.1 ha plots we observed a slightly higher effort (5.4±1.6) and a lower efficiency (13.6±7.0) for plots below 1000 m than above (4.6±1.2; 20.5±4.9). We did not observe significant differences in species richness along the latitudinal gradient.

P0826. A survey about plant association of Miankale peninsula in north of Iran

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Abstract:In this research plant associations of Miankale peninsula are presented. Miankale peninsula is situated in beside of Caspian sea (North of Iran). This Area is about 68800 hectares. The altitudes are about 21-26 meters below sea level.

The vegetation of Miankale Reserve was studied, using the Braun-Blanquet method. 123 releve in different habitats were recorded. Phytosociological data were analysed by Correspondence Factorial

Analysis (CFA) and Hierarchical Ascendent Classification (HAC) Methods. Using the Anaphyto software, 26 associations and 1 subassociation were distinguished. The distribution of associations and subassociation in this area mainly affected by chemical characters of the soil, namely Ec, Cl, CO₃H Na, Mg, Ca, SO₄. In fact the vegetation patterns are affected by these factors. And so a total of 263 species and subspecies taxa were identified based on samples collected in several years in this area.

Keywords: plant associatioc, Anaphyto software, Miankale peninsula.

P0827. A survey of species diversity in Koshk (South of Iran)

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Abstract:The area is located in southwest of Kerman, it has an area about 40000 Hectars. The region is located between 54° 32' - 56° 45' longitude and 28° 38' - 28° 50' Latitude. The lowest part of this region is about 1800 meters and the highest is about 3273 meters above the sea level. The average rainfall is about 295.5 mm, and the average annual temperature is 15.6 centigrades. In this survey the plant specimens were collected in two years (1382 & 1383). In Koshk region 200 species belonging to 47 families and 145 genera are recognized. The largest genus is *Astragalus* with 13 species. 28 families have medical values (59% total of families). 65 percent of species belong to 8 families of *Asteraceae*, *Fabaceae*, *Poaceae*, *Apiaceae*, *Boraginaceae*, *Lamiaceae* and *Liliaceae*. Therophytes with 35.5 percent is the largest life form. And so, 12 vegetation types are recognized. The *Artemisia sieberi*, *Pistacia atlantica* and *Amygdalus scoparia* is the dominant species 2/3 of the area.

P0828. Contributions of Takenoshin Nakai on the Korean and Eastern Asiatic Botany

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Takenoshin Nakai (1882-1952) was one of the most distinguished plant taxonomists in the first half of the 20th century, particularly as the authority on the Korean Flora. His *Flora Koreana* vol. 1 and 2 were published in 1909 and 1911 respectively. His monographic study of Korean woody plants, *Flora Sylvatica Koreana* in 22 volumes (1915-1939), included detailed descriptions of 1839 pages and 569 accurate plates, and can be regarded as one of the

most important early contributions to the Korean and eastern Asiatic flora. Life-long work of 37 years on vascular plants indigenous to Korea was summarized in his last paper, *Synoptical Sketch of Korean Flora*, in which 3176 species, 841 varieties and 174 formas, (4191 kinds) of vascular plants comprising 110 orders, 226 families and 968 genera were enumerated. During the period from 1907 to 1953 he had written over 600 botanical papers, and more than 300 were on the Korean flora; he also proposed more than 4000 new names. He also compiled *Notulae ad plantas Japoniae et Koreae* (1911-1935), *Notulae ad plantas Asiae Orientalis* (1937-1950), *Iconographia plantarum Asiae orientalis* (1935-1952) and *Nova flora Japonica* (1938-1951)

P0828a. Biogeography of the Afrotropical Regions

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The temperate - upper montane to alpine - floras of tropical and southern Africa contain several floral elements. Recent research has shown that those elements (e.g. *Pentstemon*, *Disa*, *Protea*) derived from the southern Cape flora most likely migrated northwards at an as yet undetermined time, and in several instances have radiated in the Eastern Highlands. Many elements are derived from the northern temperate areas, Europe and Asia. These include, for example, *Ranunculus*, *Alchemilla*, *Carex* and *Carduus*. These groups all include several African species, although the greatest diversity is found in Eurasia. Many other northern genera are represented in Africa by solitary species, such as *Rosa abyssinica* or *Primula sinensis*. We will address questions of origin and diversification of the more speciose Eurasian Afrotropical floral elements by a phylogenetic and biogeographical analysis of selected taxa, and will attempt to establish how often they reached Africa, and approximately when.

P0829. Phylogeography of *Pulsatilla vernalis* in Europe based on chloroplast DNA analysis

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Pulsatilla vernalis is an European endemic with a particular geographic distribution pattern referred to as «*Pulsatilla vernalis* type» and encompassing two ecologically distinct parts: isolated areas of European mountains' alpine and subnival zones (Alps, Pyrenees, Carpathians, Balkans and mountains of southern Norway) on the one hand and the lowlands of central Europe and southern Scandinavia on the other hand (but without expanding farther north into the boreal zone). In the phylogeographic analysis covering the whole distribution area of *P. vernalis*, direct sequencing and PCR-RFLP approaches were used to study 3 and 7 cpDNA regions respectively. 27 polymorphic sites (both length polymorphisms and nucleotide substitutions) were detected and analysed in 55 populations across Europe to assess the distribution of cpDNA haplotypes. Two main genetic lineages emerge, dominating the geographical structure of the variability and unravelling the history of post-glacial colonisation in Central and Northern Europe. A comparison of sequencing and RFLP approaches in studying phylogeographical patterns is also discussed.

P0830. Phylogeography of Japanese alpine plants

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In the high mountains of the Japanese Islands in easternmost Asia, a relatively rich alpine flora is found (ca. 500 species). The populations of each species show a disjunct distribution in these areas. In order to clarify the evolutionary history of the alpine flora of Japan, we conducted phylogeographic studies using the molecular marker of chloroplast DNA (cpDNA). Through analyses using 25 species of Japanese alpine plants, intraspecific cpDNA variations were detected in most species. Additionally, phylogenetic analyses among the populations of each species indicate an endemic clade of eleven species (*Anemone narcissiflora*, *Arnica unalaschcensis*, *Cardamine nipponica*, *Carex hakkodensis*, *C. stenantha*, *Diapensia lapponica*, *Loiseleuria procumbens*, *Pedicularis yezoensis*, *Pedicularis chamissonis*, *Primula cuneifolia*, *Tilingia ajanensis*, etc.) in the central part of

Honshu Island, Japan. From the point of view of vicariance biogeography, such similar genetic structures are explained by certain common factors. Our results suggest that the alpine region of central Honshu has served as a refugia for Japanese alpine plants during the Pleistocene period.

P0831. Differentiation and migration in the arctic-alpine diploid *Dryas octopetala* L. (Rosaceae)

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Dryas octopetala s. lat. comprises long-lived, wind-dispersed, diploid shrubs that are ecologically important in arctic and alpine areas. Here we address differentiation and migration history of the species with emphasis on the Eurasian part of its distribution area, also including some Beringian and Greenlandic populations (partly referred to as the closely related *D. integrifolia* M. Vahl). We analyzed 459 plants from 52 populations for 155 AFLP markers. Three major groups were identified. The Eurasian plants were separated into two groups, both quite distinct from the third Beringian/Greenlandic group. The Eurasian groups probably reflect isolation and expansion from two major glacial refugia. Northwestern Europe including Scotland, Iceland, and Scandinavia appear to have been colonized by a central/southern European lineage, whereas the arctic archipelago of Svalbard appears to have been colonized from the east by a northern Russian lineage occurring in the Ural Mountains and eastwards to the Taymyr area. The single populations analyzed from the Caucasus and Altai Mountains were divergent from the remaining Eurasian populations.

P0832. Do life-history characteristics determine phylogeographic patterns?

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The alpine plants *Geum reptans* and *G. montanum* differ considerably in range size, altitudinal range, habitat, successional status and mode of reproduction. Therefore, one would expect that the two species reacted differently to Quaternary climate oscillations. Owing to its high-alpine distribution on rocks and virgin soils, *G. reptans* is considered capable of in situ glacial survival, having resulted in genetic patterns related to nunatak areas. As *G. montanum* grows in late successional grasslands of lower altitude, it presumably lacked suitable habitats inside the glaciated area or at the upper level of the former ice-sheet. Therefore this species is supposed to have survived in peripheral refugia and have re-immigrated during postglacial times. Within the scope of the EU project INTRABIODIV, we compare the distributions of genetic diversity (AFLPs) of *G. reptans* and *G. montanum* sampled in 141 and 60 populations. Due to the regular sampling over the entire Alpine and Carpathian ranges of the two species, we expect to find patterns of genetic diversity that are either clumped (nunatak-related) or gradual (induced by re-immigration from peripheral refugia).

P0833. Phylogenetic analysis of species radiations using split decomposition

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Where plant species radiations are characterized by hybridization or polyploidy, complex patterns of phylogenetic relationship are expected, and bifurcating tree methods are often inadequate. In this situation, network methods may have some potential for describing patterns of diversification.

One network method useful for investigating phylogenetic incongruence is split decomposition. However, in its standard implementation this approach is limited by a conservative criterion for branch length estimation. For this reason, we have introduced a least squares optimization technique for correcting branch lengths that may otherwise be underestimated in the standard implementation of split decomposition.

We report improved divergence time estimates for splits graphs, and demonstrate the application of split decomposition analyses in the phylogeography of New Zealand alpine *Ranunculus*.

P0834. Evolutionary lineages and species delimitation in the *Saxifraga rivularis* complex

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In many arctic-alpine plant groups, reticulate histories combined with extensive range shifts have resulted in problems with species delimitation and phylogeny reconstruction. In the *Saxifraga rivularis* complex, the number of species recognized ranges from a single polymorphic one (*S. rivularis* s.l.) to several (e.g., the circumpolar *S. hyperborea*, the amphi-Atlantic *S. rivularis* and the three amphi-Pacific taxa *S. bracteata*, *S. flexuosa* and *S. arctolitoralis*). Two main chromosome numbers ($2n = 26, 52$) are known. We used molecular (AFLPs), flow cytometric and morphological data from most of the range to delimit taxonomic species and to assess their relationships. Three distinct lineages were identified based on the AFLP data (*S. bracteata*, *S. flexuosa/hyperborea*, *S. arctolitoralis/rivularis*); these lineages also differed in DNA ratios and in a combination of morphological characters. The DNA ratio data as well as the intermediate position of the *S. arctolitoralis/rivularis* lineage in analyses of the morphometric and molecular data suggest that it originated as an allopolyploid from the other two lineages, most likely in Beringia.

P0835. The geographical distribution of genetic variation in the toxic pasture weed *Veratrum album* in relation to its colonisation history

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We quantified the genetic structure of *Veratrum album* populations along a transect from the Ural and Caucasus mountains to the species' western distribution border in Portugal, using AFLP markers.

We found a highly significant correlation between the genetic and geographic distances among populations ($R^2 = 0.55$). Phylogenetic clustering and principal coordinate analysis reveal an east-west orientation in the genetic pattern and thus, provide little evidence for the importance of expected glacial refugial areas in Europe, such as the Balkan-Carpathians and the South-Western Alps. Population differentiation is significant but moderate ($F_{ST} = 0.13$). The pattern of genetic diversity seems mostly to be shaped by local habitat conditions, such as population density (positive correlation) and soil phosphorous (negative correlation).

Although pollen records document the presence of *V. album* in Europe already in the late-glacial period, our results show that the genetic structure of today's populations in Europe are mostly shaped by post-glacial re-colonisation from East-European refugia or Asia.

P0836. *Saxifraga callosa* Sm.: a complex taxon resolved by a combination of morphological and molecular approaches

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Saxifraga callosa Sm. is an evergreen perennial species distributed from Eastern Spain, through the Western Alps and the Apennines, to Southern Italy. Morphological variation among its subspecific entities and other related species (subsp. *callosa*, subsp. *catalunica* (Boiss.) D.A.Webb, var. *australis* (Moric.) D.A.Webb and *S. lantoscana* Boiss.) suggests that phenotypic characteristics are not sufficient to produce a reasonable taxonomy. Preliminary study, using ITS, AFLP and cpDNA markers, allowed a reconstruction of the genomic relationships within *S. callosa* subtaxa and related species. However, ITS analysis gave no conclusive results to assess the phylogenetic

position of them, possibly due to the recent evolution of this Alpine taxon. The application of the NewHybrids software to AFLP datasets suggested that *S. lantoscana* derived from hybridization events between *S. callosa* subsp. *callosa* and *S. cochlearis* Reichenb., living together in Maritime Alps. Introgression of genetic traits was confirmed by cpDNA markers, a simple and quick method based on length variation in cpDNA sequences.

P0837. Tracking the origin and evolution of high polyploids in *Cerastium* (Caryophyllaceae) from non-coding regions of the RNA polymerase genes

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The Arctic is one of the most polyploid-rich areas, in particular of high-level and recently evolved polyploids. The *Cerastium alpinum* group is a mature polyploid complex, with high ploidal levels dominating and no diploid progenitors known. Previous studies on morphology, isozymes, and DNA fingerprints have identified several evolutionary lineages, and the low level of cpDNA variation observed suggests recent origins and recurrent episodes of range expansions and contractions during the Quaternary glaciations. Non-coding regions of the RNA polymerase genes are now being used to identify progenitor lineages. Results from RPB2 suggest that only one functional paralogue exists in tetraploid *Cerastium* taxa. Arctic high-polyploid taxa appear to have European origins, with the tetraploid *C. semidecandrum* representing the maternal lineage and tetraploid taxa from the central and south-eastern European mountains representing different paternal lineages. However, the patterns of reticulate evolution are complicated and other putatively independent nuclear regions are needed to conclude on the origin of several individual taxa.

P0838. AFROALP - Phylogeography, glacial refugia, and conservation of the unique afroalpine ecosystem: a new project

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The unique afroalpine environment occurs in the scattered high mountains of tropical Africa like islands in a sea of lowlands. The afroalpine plants have their closest relatives not in the surrounding lowlands but in remote areas, even in the northern hemisphere. Where did their ancestors come from, how, and when? In the main part of this new project, we investigate the phylogeographic history and population genetics of 6-8 afroalpine ecological key species without obvious mechanisms for long-distance dispersal. Three populations per species have been collected in the 12 highest mountain systems of East Africa and Ethiopia, as well as from other areas. Using AFLP fingerprinting, sequencing of chloroplast DNA, phylogeographic analyses, and assignment statistics, we will determine migration rates among mountain systems, the number and location of glacial refugia, possible secondary contacts between differentiated glacial gene pools, and infraspecific diversity hotspots. In other subprojects, we address the deeper history of some afroalpine plant groups including speciation, phylogeny, and biogeography.

P0839. Phylogeography of *Arabis alpina*: What do populations from the distribution edges tell us about its evolutionary history?

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Arabis alpina is widely distributed in northern hemispheric alpine habitats and the arctic regions. Its distribution range spans an area from Central Asia to E Africa, from the Canary Islands and N Africa to Iceland and Scandinavia. We analysed 200 accessions from the whole distribution range using DNA sequence data of the nuclear ITS and the plastidic trnL-trnF region. Both data sets are mostly in congruence with each other and favour the recognition of two major lineages:

1) Central Asia, Arabian Peninsula, East African high mountains

2) All remaining areas.

DNA sequence comparisons of some selected alleles of the chalcone synthase and Ks-derived distance analysis revealed that both lineages have been separated since approximately 500.000 my. Consequently, *Arabis alpina* is not a tertiary relict in the East African mountains or the Canary Islands as hypothesized in the past, but has been greatly influenced by pleistocenic glaciation and deglaciation cycles. However, continuously distributed genetic diversity in its southern distribution area indicates fragmentation processes rather than migration.

P0840. Plant evolution on the small islands of the Russian White Sea

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Small islands and islets in the Kandalaksha Bay of the Russian White Sea are now rising. Thus, new islands are continuously appearing, these islands are settling from scratch and it is relatively easy to estimate the age of an island. We tried to find plant species that are polymorphic enough to show the differences between the mainland and island populations and the populations from the different islands. These differences could be the result of population bottleneck or even the evolutionary process on the isolated islands. Species of *Carex* (*C. salina* and *C. aquatilis* groups), *Euphrasia*, *Achillea*, *Rhodiola*, *Atriplex* and *Parnassia* were chosen for preliminary morphological analysis (~ 2200 plants were measured). In addition, geometric morphometrics methods were used for *Rhodiola* and *Atriplex* leaf shapes. We found that at least three groups show significant differences between mainland and insular populations. Some of our results are useful for the adjustment of the taxonomic boundaries in several taxonomically "critical" groups.

P0841. Biodiversity of alpine flora of Iran

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Except the interior deserts and lowlands along the Caspian Sea, Persian Gulf, c. half of Iran composed of high mountains. The Alborz along the Northern, Zagros along the Western parts of Iran and several other mountains in Khorassan and Baluchestan provide diverse mountainous ecosystems. The flora and vegetation of Iranian alpine zones are not well known. A first evaluation of vascular flora show that 675 species belonging to 205 genera and 40 families are known in alpine zones (mostly above 3000m) of Iran. Asteraceae with 119 species ranks first, followed by Fabaceae (74), Poaceae (57), Caryophyllaceae (54), Labiatae (48), Apiaceae (38), Brassicaceae (34) and Rosaceae with 32 species, respectively. *Astragalus* with 41, *Cousinia* with 20, *Nepeta* with 19, *Silene* with 18, *Oxytropis* and *Potentilla* each with 16 species are the largest alpine genera in Iran. The hemicryptophytic life form dominates in alpine zone with 73 % in Iran. The thorny cushion species are mostly dominate in the subalpine-zone. In spite of their dominance in the alpine zone their absolute number of species with 41 species is not very high. The alpine flora of Iran consisted of 53% endemics and subendemics.

P0842. Historical and contemporary processes of *Gentiana ligustica*: toward evolutionary conservation at a regional hotspot scale.

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Gentiana ligustica is a submountain neoendemic species located in the Maritime and Ligurian regional hotspot (France and Italy). A phylogeographic analysis was performed using AFLPs in 270 individuals and 28 populations. A spatial genetic structure appears in congruence with the location of the five presumed glacial microrefugia distributed along valleys. High mountains on either side of valleys correspond to contact areas between groups and constitute the major barriers to gene flows. Among the five groups of populations identified, the central group exhibit the highest Shannon diversity. The high levels of AFLP variation observed among populations and among groups, together with the lower

level of variation within groups suggest (i) a long term population insularization and (ii) the recent gene flow events over short distance. Phylogenetic analyses in relation to history and habitat diversity suggest that several populations acted as refugia during Pleistocene glaciations but did not contribute to the recolonization. These results could help to define adequate phylogeographical units and evolutionary significant units (ESUs) within this hotspot.

P0843. Phylogenetic studies in *Codium* (Chlorophyta). A molecular approach to mexican species

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Molecular phylogenetic studies in the marine green alga *Codium*, have been done with specimens from Pacific, Atlantic and Caribbean Mexico. Using information from mitochondrial LSU rDNA, we recovered clades that could correspond to 12 of the 16 nomenclatural species previously recorded in Pacific Mexico. These species are: *Codium fragile*, *C. amplivesiculatum*, *C. hubbsii*, *C. schmiederi*, *C. picturatum*, *C. brandegeei*, *C. simulans*, *C. latum* ssp. *palmerii*, *C. isabelae*, *C. giraffa*, *C. dawsonii*, and *C. setchellii*. In the other hand, topologies from Atlantic and Caribbean specimens showed only 5 clades that we recognized as species: *Codium isthmocladum*, *C. taylorii*, *C. simplex*, *C. intertextum*, and *C. decortatum*. We also combined all data in a single matrix trying to recover Pacific and Atlantic history in this genus; parsimony and likelihood analysis resulted in similar topologies showing a complex relationships where Pacific and Atlantic clades are not monophyletic.

P0844. Classical or molecular systematics in algal taxonomy? Molecular systematic and morphological investigation of the Lemnaceae (Rhodophyta, Batrachospermales)

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All freshwater representatives of red algae with uniaxial cartilaginous and pseudoparenchymatous thalli are included in the broad genus *Lemanea*. Two subgenera of this genus were distinguished, *Lemanea* and *Paralemanea*. The recently proposed elevation of these subgenera to the generic level seems to be fully justified. The 18S rDNA (SSU) sequences from 10 populations of *Lemanea* and *Paralemanea* species in the Czech Republic were used to construct phylogenetic hypotheses. Our molecular systematic features correspond with classical morphological features and validate the separation of the two genera.

However, the increasing data from natural populations show that not all the traditional diacritical features are reliable for distinguishing species. Of the four species *Lemanea fluviatilis* and *L. torulosa* appear to be well-defined, but there are no clear differences between *Paralemanea annulata* and *P. catenata*. Our investigation found no differences between *P. catenata* and *P. annulata*. On the other hand, *Lemanea fluviatilis* and *L. torulosa* appear to be morphologically well-defined species, but our results do not support this interpretation.

P0845. Green Algae From Cryptogamic Crusts, Santa Fe, New Mexico

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Cryptogamic crusts are common in desert and arid regions of the western United States. Green algae from 10 cryptogamic crusts from Santa Fe, New Mexico were isolated. The algae were grown in liquid media, and transferred to agar plates for isolation and identification. Monocultures were transferred back to liquid for DNA analysis. Genomic DNA preparation protocols have been developed by modifying Qiagen Dneasy Plant DNA Isolation Mini Kit protocols. Preparation of optimal DNA samples for sequencing and species comparison is underway. Morphological identifications will be corroborated with the DNA findings. To date, 12 isolates were found and identified to genus and 2 to species. Further isolation and identification will be carried out for the next two years.

Furthermore, cryptogamic crusts collected from the New Jersey Pine Barrens will be isolated, identified, and compared with the Santa Fe crusts' algae. Green desert algae are phylogenetically diverse, and comparison between different arid sites will allow for determination of the relationship among these green algae and their linkage to vascular plants.

P0846. A Taxonomic Study of *Hafniomonas* (Chlorophyceae) Based on Comparative Examination of Cultured Material.

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Hafniomonas is a unicellular green algal genus inhabiting freshwater. This genus is characterized by its quadriflagellated cell without cell wall/scales. Some studies of its flagellar apparatus and chloroplast multigene phylogeny indicated that this genus is one of the basal lineages of Chlorophyceae. However, electron microscopy and molecular phylogeny of cultured material has been examined in only two of eleven species, *H. reticulata* and *H. montana*. No comparative studies of multiple strains of *Hafniomonas* has been carried out.

We isolated *Hafniomonas* from six localities in Japan and compared them with two available culture strains of *H. montana*. These strains were classified into four species, *H. montana*, *H. reticulata* var. *conica*, *H. radiata* and an undescribed species, based on differences in cell shape, surface appearance of chloroplast, number of contractile vacuoles, ultrastructure of pyrenoid, stigma and muciferous vacuoles. Molecular phylogenies of both nuclear-encoded 18S rDNA and chloroplast-encoded *psaB* supported the present species classification.

P0847. *Chlainomonas* and the chlorophyte radiation

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Chlorophyta and Streptophyta comprise most of the modern green plants. Chlorophytes are thought to contain three major lineages: the Ulvophyceae, Trebouxiophyceae, and Chlorophyceae. Their common ancestor was probably a quadriflagellate unicell, possibly with a scaly covering. This is inferred from the structure of prasinophytes, the earliest divergent chlorophytes.

Recent molecular work supports a basal status for quadriflagellate taxa in the Chlorophyceae. However, few quadriflagellate taxa have yet been sampled. I add to this effort with a study of *Chlainomonas kolii*, an enigmatic quadriflagellate found in snow.

The four flagella of *Chlainomonas* and its highly unusual cytokinesis and cell covering suggest that it is a particularly early divergent chlorophyte.

Results show that *Chlainomonas* does not group with the quadriflagellates previously sequenced, but is basal in a clade including other snow taxa. Furthermore, use of *Nephroselmis* and *Chlorella* as outgroup taxa indicates that quadriflagellate relationships may be rather complex, but may be more congruent than previously thought with their variety of flagella apparatus structures.

P0848. Analysis of reproductive isolation mechanism in *Closterium*

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Closterium peracerosum-strigosum-littorale complex (*C. pslc*) is a unicellular charophyte alga. Progress of the sexual reproduction is strictly regulated by sex pheromones (PR-IP and PR-IP inducer). It was suggested that PR-IP inducer was a multi-functional pheromone, having at least three activities (stimulation of mucilage secretion, induction of sexual cell division, and induction of production of PR-IP). On the other hand, sexually isolated mating groups was known to be present in *C. pslc*, however, molecular phylogenetic relationship or variations of pheromone-encoding genes among groups had not been examined. We obtained sequences of putative orthologous genes encoding PR-IP inducer as well as the 1506 group I intron, which interrupted nuclear SSU rDNA, from each mating group. Based on the results, we will discuss phylogenetic relationships and mechanisms of reproductive isolation among these mating groups in *C. pslc*.

P0849. Additional diagnostic features of some cultured *Euastrum* species (Algae - Desmidiaceae) revealed by SEM

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Identification of *Euastrum* species is based mainly on light microscopic (LM) observations of characters such as the contour and size of the cell and the cell wall sculpturing. However, these characters are often insufficient to solve some taxonomic problems, especially when the cell has a small size and the referred characters are similar in specimens belonging to different taxa. In these cases the cell wall sculpturing observed with the scanning electron microscope (SEM) has provided additional diagnostic characters for the distinction of LM hardly distinguishable and frequently confused taxa. In this report the characteristics of 4 species of *Euastrum* cultured at ACOI (<http://www.uc.pt/botanica/ACOI.htm>) were examined under the LM and SEM. The LM examination showed that the cell wall is smooth in all of the taxa studied and that, in a few taxa, both the form and size of the cell are quite similar. Under the SEM however, the cell wall showed the presence of various sculptural elements (scrobicles, creases, pores) whose number, form and distribution are quite variable among the different taxa studied but are constant in all of the strains belonging to the same taxon.

P0850. Symbiotic organisms are stored in the diaspores

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Many species of higher plants develop symbiotic interactions with microorganisms (bacteria, algae, fungi). It is assumed that the inoculation takes place in the soil while germinating. With the help of TEM microorganisms are found in diaspores of 19 species belonging to 10 families. Microorganisms are found in such plants developing mycorrhiza as *Larix*, *Betula*, *Alnus*, *Festuca*. In each single case microorganisms were in one of pericarp or testa tissues, or endosperm, but not in the embryo. Microorganisms are often attached to cell walls at the intercellular space side. For example in the species of *Alcea* genera microorganisms in inactive form are found in exotesta cells and in intercellular spaces of pigment layer in dry seeds. In four days old seedlings live microorganisms are found on the root surfaces. In two month old seedlings microorganisms are found on the surface and within epidermal cells of the roots, just under hypocotyls. The data obtained let us suppose that microorganisms penetrating into the seeds are stored there to play active role in germination and further plant development. The revealed phenomenon we consider to be progressive.

P0851. Diversity of arbuscular mycorrhizal fungi in the Brazilian semiarid.

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The species of arbuscular mycorrhizal fungi (AMF) found in natural ecosystems, crop and impacted areas in the semiarid region of Northeast Brazil are presented. Fifty two from the 189 described species were identified (27.5% of the total). The most represented families were Glomeraceae, Acaulosporaceae, and Gigasporaceae (18, 17, 14 species, respectively). Considering that Glomeraceae has the highest number of species among the AMF, comparatively other families are better represented in the studied areas. From the total number of species, 44% of Acaulosporaceae, 57% of Gigasporaceae and only 18% of Glomeraceae were found in the Brazilian semiarid. This pattern of distribution is not similar to that mentioned for other semiarid areas, where species of Gigasporaceae were not registered. Most of the species of AMF in the Brazilian semiarid were from agrosystems (43%), followed by impacted (33%) and native areas (24%), as occurs in the other regions of the country. But this do not necessarily shows the real situation, once that most surveys were in agrosystems. Further studies are needed to increase the knowledge regarding diversity of AMF in semiarid areas.

P0852. Lichens of Oran Dosh protected area in the Lake Khubsugul National Park (Mongolia)

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The Lake Khubsugul National Park is located in north-central Mongolia in Khubsugul province. The park was established in 1992 and covers ca 9000 km². The park was nominated as a UNESCO World Cultural Heritage Landscape in 2002. Several scientists contributed to the knowledge of biodiversity of that region but the lichen flora of the area is still in need of detail investigations.

The results presented here are based on the material collected during the student expedition to Mongolia in August 2000. The lichens were collected in taiga forest and mountain tundra in Oran Dosh protected area. There were 145 taxa recorded in the studied area. Of these, 21 are new to Mongolia, e.g. *Caloplaca bohlinii*, *Lecanora zosterae*, *Lichenomphalia umbellifera*, *Trapeliopsis flexuosa*, *Xylographa vitiligo*, and further 40 are new to the lichen flora of the Khubsugul region, e.g. *Buellia geophila*, *Calicium abietinum*, *Diplotomma pharacidium*, *Fulgensia bracteata*, *Hypogymnia subobscura*, *Imshaugia aleurites*, *Japewia tornensis*, *Parmeliella triptophylla* and *Xylographa parallela*. The Lake Khubsugul National Park is a lichen-rich site worthy of further lichenological examination.

P0853. Aphylloroid fungi of the North-West of Russia

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Aphylloroid fungi are important heterotrophic components in forests ecosystems. The first information about fungi of northwestern territories of Russia was published in the 18th and 19th centuries. More intensive investigations have been carried out for last 50 years. At present more than 660 species of Aphyllorales are known in the North-West of Russia. However, degree of inventory of its different regions is very uneven: about 115 species were recorded for the Murmansk Region, 450 - for Republic of Karelia, 190 - for Arkhangelsk Region, 385 - for Komi Republic, 550 - for Leningrad Region, 90 - for Novgorod Region and 200 species - for Pskov Region. Aphylloroid fungi being mainly wood-destroying organisms are closely associated with species of trees. On investigated territory 259 aphylloroid fungi were collected on spruce, 229 - on pine, 209 - on aspen, 201 - on birch, 173 - on alder and 109 - on willow. Most interesting and rare species were found in primeval forests.

P0854. Growth forms of *Lecanora argopholis* in Kamenny Mogily reserve (Ukraine)

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In spite of Kamenny Mogily's lichens have been investigating since in 20-th in XX century (Oxner, 1927, 1961, 1962; Kovalenko, 1976, 1977), its lichenobiota is hitherto not completely inventoried. 79 lichen species, of which *Arthonia* varians is new for Reserve and Ukraine, are known for Reserve at present.

Lecanora argopholis (misapprehend as *L. frustulosa*) is analyzed, morphological variability of this species within territory of Reserve is illustrated.

Oxner (1961, 1962) describe variability of *L. argopholis-frustulosa* grope in Kamenny Mogily Reserve. After his data subfruticose growth is formed under influence of competitions, thallus development of another species. This growth form appeared to be often broken off at the basis of rocks. Vanska (1984) carries out detailed taxonomical investigation of *L. argopholis-frustulosa* grope and reported appearance of subfruticose forms together with typical in the same habitats.

Both (typical and subfruticose) thallus growth forms of *L. argopholis* are registered in Kamenny Mogily Reserve. They differs by apothecium size, namely typical form has apothecium about 3 mm diam, and subfruticose - about 0,3 mm diam.

P0855. Investigation of mycobiota in Armenia

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Armenia, situated at the heart of the Caucasus region, is an important focus of biological diversity. An unusually high fraction of Armenia's biota is endemic and the unique biological resources within Armenia are historically and economically significant. The main center for investigation of fungal diversity in Armenia is the Department of Botany, Yerevan State University. Research on Armenian fungi is currently very active with ongoing projects on the systematics, biodiversity, biotechnology, and ecology of the

Armenian mycobiota and edible mushrooms found within the country.

The Experimental Mycological Scientific Group within Department of Botany has conducted the investigations to reveal the specific composition of the Armenian fungi, inhabiting in natural and artificial ecosystems. There has been gathered more than 4000 species of micro- and macromycetes, studied their biological activity. In principal directions of mycological investigations are revealing the biodiversity, physiology and economic importance of the fungi, which are maintained in the scientific herbarium and culture collection.

P0856. Diversity and seasonal occurrence of soil fungi from certain Indian soils

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In the present investigation some cultivated soils, uncultivated soils, river soils and forest soils from India are explored for the fungal population. These fungi shows distinct diversity and seasonal variation. As far as the diversity is concerned, Deuteromycotina species were dominant from all soil types. Maximum number of species were isolated from river soil, cultivated soil, forest soil and uncultivated soil respectively. Distinct and remarkable seasonal variation was observed in the soil mycoflora. Nearly, all the isolated fungi showed remarkable seasonal variation. However some of them also exhibited regular seasonal occurrence in soil mycoflora. Minimum fungal species were isolated during summer and maximum species isolated during winter. Statistically there was a significant and remarkable correlation between pH, water holding capacity and occurrence of species from all soil types. Little less significant correlation was observed between temperature and number of species. Four species of *Chaetomium* and one variety of *Circinella* are proposed as new.

P0857. Macromycetes of small spring fens in the area of the westernmost Carpathians

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Macromycetes, vegetation and water chemistry of small spring fens (poor and rich *Sphagnum*-fens, calcareous fens and rich spring meadows) in the area of Moravian-Slovakian border were studied in 2001-2004. Our aims were to find out: a) fungi species richness; b) linkage between mycoflora composition and environmental factors; c) diagnostic fungi species for vegetation types of spring fens. Generally, species richness of spring fens was 5-21 species per locality, 95 species in total, and was equal along the pH/calcium gradient. Using CCA ordination, the variation in fungi species composition was explained best by the ordination score of vegetation. No other environmental factor significantly contributed to the CCA model. The most contrasting habitats were poor *Sphagnum*-fens (diagnostic species with the highest values of the Fischer's exact test are *Hypholoma elongatum*, *Galerina tibilocystis*, *Entoloma conferendum* and *Tephrocybe palustris*) and rich spring meadows with the best diagnostic species *Coprinus* sect. *Alachnui*. The calcareous fens without meadow character of vegetation were characterised by e.g. *Galerina heterocystis* and *Mycena epipterygia*.

P0858. Lichens of parks of Poltava town (Ukraine)

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Poltava town with population a. 400000 inhabitants is situated in the Forest-Steppe zone of central part of Ukraine. Lichens of the Poltava town parks are for the first time inventoried.

64 lichen species of 30 genera and 12 families and Mitosporic Fungi are found.

Low species diversity of lichen-forming fungi in parks of the central part of Poltava town is connected with homogeneity of trees (phorophytes), dense cover with buildings and close position to numerous motorcar roads.

Much higher number of lichen species in parks situated closely to network of artificial water reservoirs and Vorskla River at the

vicinities of Poltava town is connected with higher diversity of phorophyte species as well as higher humidity of air.

The highest species diversity of lichens (namely 37 species including rare in town taxa *Bryoria capillaries*, *Xanthoria ucrainica*, *Strangospora pinicola*, *Evernia mesomorpha* etc.) is found in town 'Dendropark' in the second group of parks.

P0859. Mycorrhizal mycobiota associated with European larch (*Larix decidua* Mill.) in Poland

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European larch is native to Europe and wide distributed in Poland, obligatory ectomycorrhizal (ECM) forest tree. Until recently our knowledge about mycorrhizal fungi colonizing root system of this tree was limited to the above-ground sporocarp inventories. Molecular methods based on polymerase chain reaction (PCR) and investigation of restriction fragment length polymorphisms (RFLP) are among the most useful approaches for more detailed identification of the fungi from mycorrhizal roots and can be target at several taxonomic levels. Two different approaches were performed to examine ECM mycobiota of European larch: morphological analysis of ECM roots and molecular techniques (PCR-RFLP and sequencing of ITS rDNA region). Numbers of individual mycorrhizal morphotypes (richness) differed depending on the age of larch trees. Young trees (1-4 years old) were mostly colonized by ectendomycorrhizal fungus *Wilcoxina mikolae* and ECM fungus - *Suillus grevillei*. Important role at the below-ground ECM community structure in larch forests fulfills *Cenococcum geophilum* and *Paxillus involutus*.

P0860. Diversity of downy mildew fungus-like organisms (family Peronosporaceae) in Crimea (Ukraine)

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Field research and study of specimens in KW, LE and LEP herbaria revealed records of 79 species of downy mildew fungi in 5 natural zones of the Crimean peninsula. Downy mildew fungi were represented by species of *Peronospora* Corda (70), *Bremia* Regel (4), *Plasmopara* J. Schröt. (3), *Paraperonospora* Constant. (1) and *Pseudoperonospora* Rostovzev (1). They were obligatory associated with 107 species of vascular plants from 69 genera of 23 families. The highest diversity of downy mildew fungi was observed in the ecotopes of steppe Crimea (51species). In 4 other natural zones, including mountain Crimea with various types of forests and south coast zone with its parks and gardens, downy mildew fungi diversity was a half of that in steppe Crimea. The high diversity of those fungi in steppe zone is caused by especially rich composition of steppants and weeds belonging to host plants of downy mildews (Dudka, 2003).

P0861. Diversity and conservation of macrofungi in Sevan National Park of Armenia

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The specially protected areas of the Republic of Armenia amounting to the total of 3110 m² (about 10% of the common territory). They have very important role in conservation of biological diversity, in particular - fungal diversity. The goal of our investigation is to study biodiversity of macromycetes of Sevan National Park. The park covers an area of 1500 m², located at the altitudes ranging from 1897 m to 2100 m above the sea level and located in the recreation and urban zone of republic, where are great anthropogenic pressure on ecosystems. As a result of our investigation detected 322 species of macromycetes from 2 subdivisions: Ascomycotina and Basidiomycotina.

Ten species of these macromycetes are marked as the candidates for the Red Book of Armenia. The list of these species is cited below: *Agaricus tabularis*, *A.xanthodermus*, *Clavariadelphus pistillaris*, *Heridium coralloides*, *Macrolepiota puellaris*, *Montagnea candollei*, *Mutinus caninus*, *Myriostoma coliforme*, *Rhodotus palmatus*, *Sarcosoma globosum*. Finally, the mapping of rare species will be presented.

P0862. Comparative mycological investigations in undisturbed soils of the Northern and Southern European taiga

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The soil fungal communities in the northern and southern taiga in Russia were characterised. All soils are rich in micromycetes; however, in the southern taiga the number of the colony formation units (58-143 thousands CFU/g) was higher than in northern (0,30-26 thousands CFU/g). Essential distinctions in fungal quantitative assessments between automorphic and hydromorphic soils have also been observed. More than 20 species have been identified in dominating complexes of micromycetes. The most frequent were species from *Penicillium*, *Trichoderma*, *Aureobasidium*, *Aspergillus*, *Acremonium*, *Chrysosporium*, *Cladosporium*, *Phialophora*. Mycelia sterilia was mainly isolated from northern soils. Cluster analysis of fungal species abundances, however, showed that automorphic soils of two taiga subzones have greater similarity than hydromorphic (peat) soils.

The research has been supported by RFBR grants (# 02-04-48870, # 03-04-06522).

P0863. Species of the genus *Setulipes* (Fungi, Basidiomycota, Agaricales) in tropical Africa

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The genus *Setulipes* Antonín (= *Marasmius* sect. *Androsacei* Kühner) represents a rather small group belonging to gymnopoid fungi. Its basic characters are following: basidiocarps small, marasmoid; lamellae well-developed; stipe insititious; basidiospores smooth, thin-walled, hyaline; cheilocystidia in the form of broom-cells or coralloid cells, rarely absent; pileipellis nonhymeniform, of diverticulate hyphae, often mixed with single broom-cells, sometimes hymeniform in primordial stages only; hyphae dextrinoid at least in stipe apex.

A list of tropical African species with their known distribution: 1. *S. rhizomorphicola* Antonín (Cameroon, Malawi, Tanzania, probably also Burundi). 2. *S. affibulatus* Antonín (Burundi, Democratic Republic of Congo, Malawi, Nigeria, Tanzania). 3. *S. brevistipitatus* Antonín (Cameroon). 4. *S. curvistipitatus* Antonín (Burundi). 5. *S. congolensis* (Beeli) Antonín (Democratic Republic of Congo). 6. *S. kisangensis* (Singer) Antonín (Democratic Republic of Congo). 7. *S. hakgalensis* (Petch) Antonín (Democratic Republic of Congo, Malawi, Tanzania, also Sri Lanka, Lesser Antilles, South America).

P0864. Taxonomy and importance of *Hohenbuehelia*, *Resupinatus* and *Pleurotus* in *Meloidogyne javanica* control

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Were collected and identified species of *Hohenbuehelia*, *Pleurotus* e *Resupinatus* (fungi, Basidiomycota) in southern Brazil and their action as predators against *Meloidogyne javanica* assayed. The fungi were cultivated in PDA and the nematodes were inoculated from a tobacco isolate cultivated in roots of tomato plants (*Lycopersicon esculentum*) var. gauchó. Were identified 3 species of *Pleurotus*, 10 of *Hohenbuehelia* and 2 of *Resupinatus*. *Pleurotus cystidiosus*, *Resupinatus silvanus*, *Hohenbuehelia unguicularis* and *Hohenbuehelia singeri* are new references to Brazil and one species in new to science. In the tests against *Meloidogyne javanica*, the better predator was *Hohenbuehelia portegna* (85,64%), followed by *Pleurotus ostreatus* (76,44%). In greenhouse, *Hohenbuehelia paraguayensis*, *H. portegna*, *Pleurotus cystidiosus*, *P. ostreatus* and *P. ostreatoroseus*, isolated or in cocktail, were efficient in controlling *Meloidogyne javanica*. *Pleurotus ostreatus*, *P. ostreatoroseus* and the cocktail reduced 70% the gall numbers in roots and *Hohenbuehelia portegna* and *H. paraguayensis*, reduced 50% and 40%, respectively.

P0864a. Macromycetes of beech stands in Central Slovakia and their response to decrease on air polluting load over the last years

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The aim of this study was to evaluate how the species composition of macromycetes communities can contribute to the ecological stability of beech ecosystems. We examined quantitative data about macromycetes from three permanent research plots with various impacts of airborne pollutants generated by an aluminium plant in Central Slovakia. This topic was studied in the vegetation periods 2003 and 2004. We have found balanced values of abundance of fruiting bodies and species dominance on all the examined plots. We can conclude that the distribution of tree parasites decreased with decreasing imission load. We have also found relatively equal numbers of lignicolous saprophytes and terrestrial saprophytes on research plots. The negative influence of airborne pollutants on ectomycorrhizae forming species was observed at the beginning of the 1990s. The current assessment of their abundance and dominance allows us to conclude about an increase in ectotrophic forest stability.

P0865. Diversity in virulence of *Blumeria graminis* f. sp. *tritici* from five regions of Ukraine

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The virulence and race structure of the populations of powdery mildew fungus (*Blumeria graminis* f. sp. *tritici*) from five regions of Ukraine have been examining since 1998. This regions (Kyiv, Kherson, Ternopil, Zhitomir and Ivano-Frankivsk districts) have differences in soil-climatic conditions, levels of chemical pollution and cultivating varieties of wheat. It was found that populations of powdery mildew fungus from five regions were different elements of the same population. The maximum genetic distance between these elements was 0.06. The majority of 40 identified fungus races possessed low virulence. The most races diversity and high virulence of pathogen was determined in Ivano-Frankivsk district. It may be associate with chemical pollution of sown areas or with migration of fungus from western countries. Diversity in virulence (available virulence genes and their number) caused by efficiency of resistance genes.

P0866. Phylogenetic relationships between *Puccinia pachyderma*, *P. wolgensis* and *Aecidium gageae* (Uredinales) based on ITS sequence data

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Puccinia pachyderma is a microcyclic rust species on *Gagea* spp. (Liliaceae) native of Europe and Asia. This species has erumpent telia with thick-walled teliospores. Another Eurasian rust species on *Gagea* is the anamorphic *Aecidium gageae*. Teliospores of *P. pachyderma* are morphologically almost identical with those of *Puccinia wolgensis* on *Stipa* spp. (Poaceae), which is, like nearly all grass rusts, supposed to be heteroecious. In order to find possible relationships between the three species (*Aecidium gageae* an aecial state of *P. wolgensis*? *P. pachyderma* a reduced microcyclic species evolving from *P. wolgensis* according to Tranzschel's law?), we sequenced rDNA (ITS1+5.8S+ITS2) extracted from herbarium specimens. Phylogenetic analysis indicates that there is no relation between *P. pachyderma* and *P. wolgensis* but strong similarity between *A. gageae* and *P. pachyderma* with only four bp difference. We assume *Puccinia pachyderma* to be an opis form, i.e. with spore states 0, I (= *Aecidium gageae*), III, and IV.

P0867. Smut fungi from northwestern China

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There are 110 known species belonging to eighteen genera of smut fungi known from Northwestern China until the end of the

year 2004. These species were collected from the Shaanxi Province, the Gansu Province, the Qinghai Province, the Ningxia Hui Autonomous Region and the Xinjiang Uygur Autonomous Region by the author and her colleagues during several mycological expeditions. The study is also based on examinations of specimens deposited in the Mycological Herbarium of Chinese Academy of Sciences (HMAS). This number occurs in about half of the species of smut fungi existing in China. These 10 endemic species are considered to Northwestern China occurring in four genera, namely *Anthracoidea filifoliae*, *Anthracoidea kanasensis*, *Anthracoidea microspora*, *Anthracoidea pygmaea*, *Anthracoidea shaanxiensis*, *Anthracoidea striata*, *Sporisorium reticulatum*, *Thecaphora sphaerophysa*, *Urocystis helanensis* and *Urocystis qinghaiensis*.

P0868. The diversity of Ganodermataceae in Hainan province, south China

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Ganodermataceae species called "Lingzhi" in China, it has been used as Chinese traditional medicine and food from ancient China, China is a country in possession of most ganodermatoid fungi. Many people are very familiar with "Lingzhi" and there are many myths from ancient China. "Lingzhi" is an emblem of good fortune and prosperity in Chinese people. The diversity of Ganodermataceae has been investigated; Ninety-eight species have been reported from China belonging to four genera. (Zhao & Zhang, 2000)

Hainan Island is located in the tropics and is a southmost province in China. It is characterized by high temperature, humidity and so much rainfall throughout the year. Hainan has the highest diversity of plants, thus influence the richness of fungal resources. Particularly, there was a high diversity of Ganodermataceae, species of the Ganodermataceae were founded in Hainan province is over 79 % of the total in China. Seventy species of Ganodermataceae in Hainan were reported.

Six species of "Lingzhi" have been successfully cultivated and widely used as Chinese traditional medicine and health food in China and southeast Asia.

P0869. Analysis of genetic diversity in *Colletotrichum acutatum* using rDNA-ITS sequences

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The ascomycete fungus *Colletotrichum acutatum* is a cosmopolitan plant pathogen, responsible for diseases known as anthracnoses which cause important economic losses particularly in crops such as citrus, strawberry, olive, peach, almond, lupin and several ornamentals. This taxon was created by J.H. Simmonds in 1965 and since then several plant pathogens were assigned to it. Currently *C. acutatum* encompasses a high level of genetic diversity, leading some authors to define new species from within *C. acutatum* populations. In this work we have analysed the genetic variability of *C. acutatum* using rDNA-ITS sequences obtained by us and other authors around the globe and compare them with the host range and morphological characters of a selection of isolates. At least eight genetic groups can be defined within *C. acutatum*, some of which are geographically and/or host confined, while others can be found world wide in numerous hosts. Results suggest that *C. acutatum* is a single taxon but intra-specific differentiation could be useful to address the high degree of genetic diversity recorded within this pathogen populations.

P0870. The role of mycorrhizae in heavy metals tolerance of *Erica andevalensis*

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It is generally known that mycorrhizae plays an important role in heavy metal tolerance in Ericaceae. We have assessed the presence of mycorrhizal fungi infection in the species of Ericaceae (including *Erica* and *Calluna* genus) of the province of Huelva (Spain). In the piritic belt of Huelva, there is an endemic heather named *Erica andevalensis*, which is able to grow in highly polluted environments. This species grow in the bare spoil heaps resulting of the pyrite mining extraction activity that was frequent in the zone and in the river bank of the Odiel and Tinto rivers, which run

through the area. High heavy metals concentration and low pH values (up to 2) have been detected in the area.

Our results show that *E. andevalensis* has a similar percentage of mycorrhizal fungi infection (lower than 10%) when compared with other analysed species from non-contaminated places. This result could indicate that the heavy metal pollution is not related to the abundance of mycorrhizal fungi infection in *E. andevalensis*. In this case, a non-mycorrhizal mechanism should be involved in heavy metal tolerance. This fact will be the subject of further research in this peculiar species.

P0871. Phylogenetic hypothesis of Microbotryum

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Based on molecular studies, we discuss the phylogenetic relationships in the phytoparasitic family Microbotryaceae. The most prominent members of this family are the anther smuts of the genus *Microbotryum*, which form their sori in the anthers of Caryophyllaceae and are transmitted by pollinators. Besides the Caryophyllaceae, species of *Microbotryum* parasitise Asteraceae, Dipsacaceae, Gentianaceae, Lamiaceae, Lentibulariaceae, Onagraceae, Polygonaceae, and Portulacaceae. In addition to anther smuts, there are also species forming their sori in the gynoeceum/seeds, the whole inflorescence, leaves or the pedicels. Our analyses of the ITS show that parasitism of *Microbotryum* on Polygonaceae is the ancestral state for these parasites. We confirm the monophyly of the caryophyllaceous anther smuts and show that separation in European and North American anther smuts occurred before their radiation. Sorus formation in anthers itself has evolved at least twice.

P0872. Does the phylogeny of the smut genus *Entyloma* relate to the evolution of host plants?

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The "white smut" *Entyloma* is easily recognized by the white dense leaf spots developed during teliospore formation. Although the 170 *Entyloma* species parasitize a phylogenetically wide range of dicots, about 85% of the species occur on Asterids or Ranunculales whereas single species show strict host specificity on the level of genera. Patterns where smut groups are restricted to certain host clades are common, but are not necessarily the result of cospeciation as the major speciation mode of the parasites. Admittedly, recent analyses comparing *Entyloma* and respective host phylogenies showed significant codivergence of parasites and hosts, but on the basis of a phylogenetic analysis of a relatively small dataset compiled from literature.

Here we reexamine the evolution of *Entyloma* with respect to the phylogeny of hosts on the basis of an enlarged molecular sampling and revised presence-absence data. The results are interpreted with respect to general parasitological rules including current theories of host-parasite evolution.

P0873. Arbuscular mycorrhiza in primary succession on coal mine spoil banks

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Arbuscular mycorrhizal fungi (AMF) are an integral part of natural communities, but little attention has been paid so far to the establishment of arbuscular mycorrhiza in newly formed ecosystems.

Mycorrhizal associations of five pioneer plants with different mycotrophy are investigated in microcosm experiments simulating conditions of primary succession on a freshly levelled coal mine spoil bank. The aim of the study is to describe how mycorrhiza influences the coexistence of the pioneer plants and vice versa, how plants with different mycotrophy affect the colonisation of the spoil bank substrate with AMF.

Radiation of extraradical mycelium from intact root systems represented the most effective agent of AMF colonisation of the spoil banks in comparison with AMF proliferation from isolated propagules such as spores. The pioneer plant species differentially supported the development of AMF populations in the spoil bank substrate. The specific effects of plant species on individual AMF species in community were studied by PCR with specific primers.

The research is supported by grant No 526/04/0996 (Grant Agency of the Czech Republic).

P0874. Host preferences of arbuscular mycorrhizal fungi?

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The aim of this study was to characterize the communities of arbuscular mycorrhizal fungi (AMF) in the roots of different plant species and to determine whether possible differences can be explained by host preference or spatial heterogeneity.

Two field sites in Switzerland were analyzed, a lowland and a subalpine species-rich grassland. In each site, four and nine plant species were sampled, respectively. The roots were analyzed by nested PCR of a region of the ribosomal RNA genes, RFLP analysis and sequencing. Sequences of AMF were analyzed phylogenetically and used to define monophyletic sequence types. The differences among AMF communities in different root samples were evaluated using canonical correspondence analysis.

Preliminary data show that the AMF communities in the lowland and the subalpine site were significantly different from each other. Spatial variability was not significant within any field site. Significant differences among AMF colonizing different plant species were observed in the subalpine field site. Two co-occurring gentian species (*G. acaulis*, *G. verna*) hosted very diverse but distinct AMF communities.

P0875. Studies on effect of fungal pigments on seed discolouration

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ABSTRACT

Association of fungi with developing seeds in the field and with the matured seeds under ill storage has been found to cause biodeterioration of the seeds. This may cause various types of the abnormalities in the seeds. These abnormalities include loss in food content, toxification of seed chemicals, loss in germinability, rotting and seed discolouration.

Among the above types of seed abnormalities, seed discolouration has been considered to be significantly important on the account of its distraction effects. Seed discolouration can be attributed mainly due to presence of coloured mycelia, conidia, conidiophore of the associated fungi, which produced coloured pigments, which can be retained in the staining of the seed coats. Discoloured seed mycoflora of few commonly growing cereals, Pulses & oilseeds were isolated to pin point the fungi responsible for seed discolouration.

Crude fungal pigments were tasted for its biological properties. Effect of fungal pigments on seed germination, fungal spore germination and on bacterial growth was studied. It was observed that the fungal pigments were antibacterial and antifungal in nature.

P0876. A new *Ophiostoma* species from oak in Europe.

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Ophiostoma stenoceras, a well-known sap staining fungus occurring on hardwoods and conifers in Europe and North America is characterized by a micronematous *Sporothrix* anamorph. Recent phylogenetic studies have shown that isolates of *Ophiostoma stenoceras sensu lato* include several closely related species. Isolates from oak in Poland and Hungary, which have previously been referred to as *O. stenoceras* as well as isolates of *S. inflata* were considered in this study. Sequence data for the ITS/5.8S gene regions of rDNA operon and partial α -tubulin gene were used to characterize isolates. Phylogenetic analyses showed that the isolates reside in a well resolved clade, separate from those in the *O. stenoceras*-complex. In addition, distinct morphological differences in teleomorph and anamorph structures were found between isolates from Poland and Hungary and those in the *O. stenoceras*-complex. The fungus thus represents a new species, which is currently being described as *Ophiostoma dentifundum*. This species is phylogenetically related to isolates of *S. inflata*, which consist of four well-defined groups based on both morphology and DNA sequence phylogeny.

P0877. Dynamics of peroxidase complex activity in pathogen system *Claviceps purpurea* - *Secale cereale*

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The strains of fungus have different biosynthetic alkaloid capabilities and predominantly produce a certain peptide alkaloid. In submerged cultures enzyme activity is lower at ergocristine strains and higher at cultures obtained from ergotamine and ergocryptine sclerotia. Peroxidase activity of sclerotia is strong at beginning of ontogenetic development, and decrease to the end of sclerotia development. The parasite influences peroxidase activity in host plants. The alkaloid accumulation in sclerotia and intensity of peroxidase activity there are in relation of inverse proportionality. The alkaloid complex influences the peroxidase activity in plant. Comparatively with control, enzyme activity level is lower in parasitized plants by ergotamine sclerotia and higher in parasitized plants by ergotamine sclerotia. Peroxidase isoforms number in host plants varies depending on age and predominant alkaloid in sclerotia which growth on *Secale cereale*.

P0878. Genetic variation in the foliicolous *Porina epiphylla* group

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More than one third of the species of the lichen family Porinaceae occur obligately on living leaves. Members of the species-rich genus *Porina* grow abundantly in tropical rainforests. Highly adapted to the ephemeral nature of their substrates, foliicolous lichens show significantly shorter lifecycles compared to lichens of other substrates. Therefore they are interesting objects to analyse evolutionary processes. In our studies we focused on selected species of the *Porina epiphylla* group collected in lowland rainforests in Costa Rica. All of the investigated species produce abundantly fruiting bodies, and in one of them, *Porina alba*, also vegetative diaspores are common. Sequences of the mitochondrial SSU rDNA were analysed using phylogenetic methods and haplotype networks. The genetic variation of various species is analysed with respect of geographical and ecological parameters, including symbiont selectivity patterns.

P0879. Terricolous *Buellia* species in Southern Africa

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Buellia species growing on soil in xerophilic, rather alkaline situations which have more or less lobate growth-forms are currently merged in the *Buellia epigaea* group. Specimens of soil-growing *Buellia* were collected in semi-desert, lichenologically very poorly investigated, areas of Southern Africa. They were found to be morphologically very similar to species of the *Buellia epigaea* group and have been studied in greater detail for the first time.

Anatomical, chemical and molecular characters (mtSSU rDNA sequences) have been analysed to elucidate the relationship of these taxa. Four taxa could be distinguished from the already known species. These do not form a homogenous group. Preliminary molecular results show that two of the southern African taxa are nested within the *Buellia epigaea* group, comprising Australian taxa and species of the northern hemisphere, whereas the other two do not seem to be so closely related.

An outline of specimens collected from xerophilic sites from various parts of the world, with a special focus on the southern hemisphere, will be given. Thus, several other soil-growing species will be included in the study.

P0880. Contribution to the genus *Cladonia* in the Iberian Peninsula. III. Supergroup *Perviae*

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Phylogenetic studies of the genus *Cladonia* suggest a provisional supergroup *Perviae*, defined by axils usually dilated to gaping funnels, brownish apothecia and the presence of β -orcinol depsides. It is constituted mostly by the old section *Perviae* and section *Strepsiles*. A complete morphological, chemical and biogeographical study of six species of this supergroup is

presented to complete the knowledge of the genus in the Iberian Peninsula, as part of the project Spanish Lichenological Flore (BOS2001-0869-C04-04). In many cases the Iberian Peninsula (Portugal and Spain) represent the southern outpost in their European distributions: a) species distributed in the Eurosiberian Region which rarely reach the Mediterranean Region: *C. parasitica* and *C. crispata* var. *ceptrariiformis*; b) species with the southern limit in the Sistema Central mountains: *C. cenotea* and *C. strepsilis*; c) species with the southern limit in Sierra Morena mountains: *C. glauca* and *C. squamosa*.

P0881. Calicioid lichens and fungi of Hungary

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The recent revision of Hungarian calicioid lichens and fungi based on more than 200 literature records and 300 herbarium specimens kept in BP, EGR, hb. Seaward (Bradford), JPU, S, SZE, SZO, UPS, VBI and W confirmed the presence of the following 6 genera (with number of species in brackets): Calicium (7), Chaenotheca (10), Cyphelium (2), Microcalicium (1), Mycocalicium (1) and Sphinctrina (2). Compared to Versegly's 1994 compilation Chaenothecopsis (6), Phaeocalicium (1), Sclerophora (1), Stenocybe (1) and Thelomma (1) are reported as new genera to the Hungarian lichen flora. Chaenothecopsis debilis, Chs. pusiola, Chs. rubescens, Sclerophora nivea, Sphinctrina tubaeformis, Stenocybe pullatula and Thelomma ocellatum are recently recognised species from Hungary. Characterisation of genera and species and distribution maps of the most frequent species are presented.

P0882. The high-alpine lichens of Dzherginsky natural reserve (Northern Pribaikalie)

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Dzherginsky reserve is situated in northern part of Baikal basin on slopes of South Muysky and Ikatsky ranges (54°56' - 55°27' S.L., 111°11' - 111°58' E.L.) on altitude 800-2500 m. Peaks ("goltsy") are massive and gentle, windswept snow-free with talus and stone field. 108 lichens species are found in High zone. Arctic-Alpine species (57%) and widely spread lichens (99%) predominant. Dry lichens and dryas tundra dominate in plant cover of ridge. Community's with Alectoria ochroleuca, Thamnolia vermicularis, Cetraria laevigata, Bryocaulon divergens and Bryoria nitidula occur on scree soils. Caloplaca sibirica, C. jungermanniae, Ochrolechia upsaliensis, Mycobilimbia berengeriana grow on plant remnants. On excess moist substrate dwarf shrubby, sedges, mosses tundra develop. Mosses and plant remnant occupied by Lecanora epibryon, Rinodina roscida, Bryonora castanea, Pertusaria christae, P. trochiscea. Stones and rocks cover by different lichens: Asahinea chrysantha, Umbilicaria and Lasallia species, Ophioparma ventosum, Rhizocarpon and Porpidia species, Boreoplaca ultrafrigida, Clauzadeana macula, Fuscidea austera, Miriquidica griseoatra.

P0883. Species delimitation in the lichen subgenus Neuropogon

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Species of the lichen genus *Usnea* subg. *Neuropogon* have their centre of distribution in polar regions of the Southern Hemisphere. Morphological and chemical variability is poorly understood and several asexual taxa with uncertain relationships to fertile taxa occur in the group.

Based on molecular data of three nuclear genes (IGS rDNA, ITS rDNA, RPB1) and one mitochondrial gene (COI) we study the species delimitations of three groups (288 ingroup specimens) within Neuropogon: 1) the *U. antarctica*/*U. aurantiaco-atra*-group, 2) the *U. sphacelata*/*U. trachycarpa*/*U. subantarctica*-group and 3) the *U. perpusilla*-group. Each group consists of sexual and asexual individuals. In addition to the molecular analyses, thallus

morphology and anatomy and secondary chemistry were analysed.

Phylogenetic, phenotypic and geographic species criteria were applied to test the species recognition in the groups. In addition, cohesion species recognition according to Templeton was employed and the results of the different approaches compared. Congruence in species recognition between the different methods was interpreted as indicating isolated evolutionary lineages.

P0884. Diversity of Georgia's Lichens

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Georgia's lichen flora has been studied since the beginning of the XIX century. During the 200 years separate regions of the country were studied by European, Russian and Georgian scientists: E. Acharius, F. Ruprecht, J. Steiner, A. Elenkin, E. Vainio, A. Jatta, V. Pakhunova, Ts. Inashvili, N. Chelidze, etc. By this time more than 730 species (987 taxa including subspecies, varieties, forms) are recorded in Georgia. These data do not reflect the actual lichenological richness of the country, as long as some regions are not yet investigated in this aspect. Therefore, the actual number of species must be much greater.

The paper includes an introduction on the history of lichenological research in Georgia, presents a complete checklist of Georgia's lichens with the distribution, ecology (habitats and distribution according to altitudes), frequency of occurrence, year of collection and the name(s) of the collector(s) indicated for each species. A discussion concerning the taxonomic, morphological, ecological and coenotic analyses of the presented checklist, appropriate diagrams and a vegetation map with indicated lichen vertical distribution is also provided.

P0885. 'Four' morphospecies within *Parmelina quercina*: geographic distribution

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Parmelina quercina is one of the oldest parmelioid names, and was proposed by Willdenow in 1787 to describe specimens from Germany. This lichen has a disjunct area, growing in zones with a Mediterranean climate in North America (California) and Europe, and has also been reported from similar climatic areas of South Africa and Australia. We studied specimens from Europe, America and Australia. Molecular analyses based on nrITS, nrLSU and mtSSU sequences show four monophyletic groups. The spores and epicortex features are correlated to the topology of the phylogenetic tree. The Australian specimen clearly separates from the other samples. The European samples split into two groups. The first one has wider spores and a maculated (fenestrated) epicortex, matching what was described as *P. carporhizans* by Taylor in 1847 based on Canary Islands specimens. The second group has narrower spores and lack maculated (fenestrated) upper surface. The latter is closest to the *P. quercina* description of Willdenow. The North American samples form another group and have fenestrated epicortex. Both the American and Australian specimens should be granted the status of new species.

P0886. Is *Xanthoria parietina* reliable model taxon?

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Detail revision of material called as '*Xanthoria parietina*' with involving anatomical and biochemical characters shown it to be very heterogeneous. A number of new taxa, namely *X. dissectulata* and *X. bonae-spei* from South Africa, as well as *X. elixii* and *X. streimannii* from Australia is recently described.

Detail comparative morphometric study of collections of *X. parietina* material from Europe and Asia is carried out during this study. Some specimens are similar with *X. steineri* by sporae size but they have different thallus morphology. Also, some specimens have similar morphological characters with *X. parietina* but they have different anatomical characters (including the spore length and width). Reinvestigation of the type of *X. parietina* with aim to check all characters used in modern taxonomy of xanthoroid lichens is in urgent need.

Determination of *X. parietina* material, especially from different continents including Europe and Asia, should be done with great care and with including anatomical characters. *X. parietina* is

confirmed only by European material indeed. Asian Near East material of '*X. parietina*' shows characters of *X. steineri* aggr.

P0887. The New Records of Lichen from Iran

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The lichen taxonomy is a deep gap in the taxonomic studies for Iranian vegetations. In the course of a Ph.D thesis on the lichen flora, collection, number and identification of the available lichens in khorasan province were accomplished in the aim of preparing the references specimens for comparison. From phytogeographical point of view, Khorasan is in the Irano-Touranian vegetation region and located in the northeast of Iran. It's distinguished by the presence of mountain ranges that show much more symmetry and regularity. Geologically, the predominant series of the main Alborz- lower Jurassic and Cretaceous- continue uninterruptedly to form the main massifs of Khorasan.

Morphological and chemical studies were done with using microscopical standard techniques and spot tests with the current reagents.

10 species of lichens in the altitude ranging between 800-2200 m with

40 percents of the average daily humidity, were identified and are related as new species for Iran.

P0888. The lichen flora of Termessos National Park (Antalya) in southwestern Anatolia

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This research aim to determine the lichen flora of the Termessos National Park. Termessos National Park is famous for its ancient city and biological diversity in Antalya, Southwest of Turkey. It was founded on a natural platform on top of Güllük Mountain, soaring to a height of 1665 meters from among the surrounding travertine mountains of Antalya, which average only 250 meters above sea level. Due to the area was protected, is far from the city center, has different topography and plant cover, the lichens live densely in this area. Until this research, there wasn't any lichen records from Termessos National Park.

Total 161 taxa (152 species, 4 subspecies, 5 varieties) were determined from 1114 pieces of lichen samples gathered in the area research done between the dates March 2002 and September 2003. All of 161 taxa for Termessos National Park, 93 of total taxa for Antalya Province, 43 of total taxa for Mediterranean Geographical Region, 30 of total taxa for Turkey's Mediterranean Phytogeographical Region, 11 of total taxa are new records for Turkey.

P0889. Phylogeny of *Dicranoloma* (Bryophyta, Dicranaceae), based on ITS and morphology

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Previous cladistic analyses into the delimitation of and higher level relationships within the Dicranaceae, based on chloroplast sequences, have resolved *Dicranoloma* and *Dicranum*, each with a few satellite genera, as separate monophyletic groups. The present study aims to reconstruct phylogenetic relationships within the *Dicranoloma* clade, using the more quickly evolving nuclear ITS region. Sequences of the entire ITS region have been obtained from specimens representing all Australian and New Zealand species and as many as possible non-Australasian species of *Dicranoloma*. Cladistic analysis of ITS sequences provides excellent resolution between the specific and generic levels and divides the *Dicranoloma* clade into two major subclades. ITS results are largely corroborated, if not independently obtained, by morphological data. Several species in both subclades show Southern Hemisphere disjunct distributions and make good candidates for future comparative phylogeographic studies.

P0890. Phylogeny of Lejeuneaceae (Jungermanniopsida) based on the cp *rbcL*, *trnL-F* and *nrITS*.

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Lejeuneaceae are the largest family of liverworts with about 1000 species in ca. 90 currently accepted genera in two subfamilies. Although morphological studies have gone a long way to help understand the phylogeny of Lejeuneaceae, they have been hampered by the lack of stable morphological characters. Ongoing molecular-phylogenetic studies based on the chloroplast markers *rbcL*, *trnL-F* and the nuclear marker ITS suggest that a reconsideration of the classification of Lejeuneaceae into 3-4 subfamilies is needed. *Metzgeriopsis* is resolved as a member of the *Cololejeuna* clade, *Taxilejeunea* and *Neopotamolejeunea* are nested within *Lejeunea*, and *Phaeolejeunea* is resolved as a member of the Ptychanthoideae. Molecular data indicate that the *Cheilolejeunea-Omphalanthus-Aureolejeunea* complex is in need of a thorough revision leading to a broader genus concept for this clade.

P0891. A taxonomic revision of *Herbertus* (Jungermanniopsida: Herbertaceae) in tropical America based on nuclear and chloroplast markers and morphology.

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Taxonomy of *Herbertus* is notoriously difficult because of the lack of stable morphological characters. Currently, eleven species are accepted for tropical America. Phylogenetic analyses of the chloroplast marker *trnL-F* and the nuclear ITS1-5.8S-ITS2 region resolves the neotropical phenotype spectrum in two robust clades. Based on the molecular topology and morphology, a new concept is proposed for neotropical *Herbertus*, leading to the recognition of two species only. The neotropical *Herbertus juniperoideus* is subdivided into four subspecies (ssp. *acanthelium*, ssp. *divergens*, ssp. *pensilis*, ssp. *juniperoideus*). *Herbertus sendtneri* (*H. subdentatus* auct.) occurs in the Neotropics as well as in Europe and Asia. The species is nested in a clade with Holarctic, Asian and African taxa. Without much doubt, the morphologically similar *H. dicranus* is restricted to Asia and Africa.

P0892. The family Metzgeriaceae (Hepaticae) in the Tropical America.

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The family Metzgeriaceae is represented in the Tropical America by one genus and 57 species. The species are characterized according to width of thallus, number of laminal cells from midrib to margin, of dorsal and ventral epidermal cells in the midrib, of medulla cells, size of laminal cells, verruculosity of the cuticle, types and disposition of hairs in the thallus, gemmae, and sexuality. They are mostly epiphytes in humid, submontane to upper montane tropical forests, between 500-4000 m. Most of the species are restricted to Tropical America and the highest species diversity is found in the Northern Andes, followed by the West Indies, Central Andes and Southeastern Brazil. In dry regions few species are found. About 13 % of the species are limited to undisturbed forest and 29,7 % are included in one of the IUCN categories: extinct (1,7 %), critically endangered (1,7 %), endangered (3,5 %), vulnerable (14 %), or data deficient (8,8 %). Fifteen new synonyms are proposed.

P0893. Systematics and distribution of Mexican *Grimmia* (Bryopsida)

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Although broadly distributed in Mexico, the genus *Grimmia* is usually found between 1200 and 4600 m in elevation in three main areas: the northwestern states (Baja California to Chihuahua and Durango), along the Neovolcanic Belt, and in the dry areas of central Mexico. It is represented there by 18 species, sixteen of which are widely distributed in the world and two are endemic to the country. A preliminary cladistic analysis of the genus indicates that most Mexican species are grouped into three main clades that show no relationship to geography or to existing taxonomic schemes. Because of their broad range and uneven Mexican distribution, it is suggested that the species represent ancient lineages that spread at various times from northern areas, southward through the western Cordillera, and into the mountains of central Mexico.

The distribution of the Mexican endemics, *Grimmia involuocrata* Card. and *G. pulla* Card., is under study to determine the relationship to other species and their ranges.

P0894. A taxonomic study of the genus *Syntrichia* Brid. (Pottiaceae, Musci) in Europe

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The genus *Syntrichia* Brid. (Pottiaceae, Musci) is taxonomically revised in Europe based on the study of more than 3000 specimens from 62 herbaria, examination of type material and an extensive review of relevant literature. Nineteen species and five varieties are recognized: *Syntrichia calcicola*, *S. caninervis* var. *caninervis*, *S. caninervis* var. *abanchesii*, *S. caninervis* var. *astrakhanica*, *S. caninervis* var. *gypsophila*, *S. echinata*, *S. fragilis*, *S. glabra*, *S. handellii*, *S. laevipila*, *S. latifolia*, *S. minor*, *S. montana* var. *montana*, *S. montana* var. *calva*, *S. norvegica*, *S. papillosa*, *S. papillosissima*, *S. princeps*, *S. rigescens*, *S. subpapillosissima*, *S. ruralis* var. *ruralis*, *S. ruralis* var. *ruraliformis*, *S. sinensis* and *S. virescens*. A key for identification of species is provided, and for each one synonymy, description, illustration, distribution, diagnostic characters and new records are given. Five new synonyms are proposed. Lectotypes for 13 species are newly selected. Finally the name *Syntrichia aciphylla* var. *calva* is excluded from the *Syntrichia* genus.

P0895. Taxonomic studies on the genus *Cololejeunea* (Hepaticae, Lejeuneaceae) in Asia.--Revision of *Cololejeunea* in China

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Lejeuneaceae is a pantropical genus, containing over 90 genera currently accepted. It is the largest family in Hepaticae. *Cololejeunea* (Spruce) Schiffn., the second largest genus of Lejeuneaceae, is the most dominant genus of epiphyllous liverworts. Different from most other genera of leafy Hepaticae, the generic and subgeneric concept of *Cololejeunea* has been long disputed. In China, *Cololejeunea* is the largest genus of Hepaticae. However, the genus has not been revised. The present paper presents a taxonomic revision. Four traditional subgenera are accepted: subg. *Aphanolejeunea*, subg. *Chlorolejeunea*, subg. *Cololejeunea*, subg. *Leptocolea*. Subg. *Pedinolejeunea* and *Chlorocolea* are proposed as synonyms of subg. *Chlorolejeunea*. *Cololejeunea calcarea*, *C. amoena*, *C. plagiophylla*, *C. reinecheana*, and *C. yoshinagana* is excluded from China. *Cololejeunea shimizui* subsp. *shihuishanensis* were moved to *Chondriolejeunea*, and it is a synonym of *Chondriolejeunea chinii*. A total of 72 species are recognized in China. A key to subgenera and species in China is provided. Taxonomic notes, distribution, and habitats of each species are presented.

P0896. Tree fern phylogeny revealed by the simultaneous analysis of 8kbp of plastid DNA sequence data

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Tree ferns are a large monophyletic group of mainly tropical leptosporangiate ferns, comprising approximately 600 species in seven families, the largest being Cyatheaceae (scaly tree ferns). Despite the name, not all tree ferns are arborescent: Metaxycaceae have long creeping rhizomes and Hymenophyllopsidaceae are diminutive. Although tree ferns hold a prominent position in fern phylogeny as sister group to the polypods (>80% of living fern species), relationships among major tree fern lineages have been elusive until now. Here, using a taxonomic sampling of 70 species, we reveal tree fern relationships based on 8 kbp of plastid DNA sequence data from coding and non-coding regions. In previous single-gene analyses, tree ferns were shown to have remarkably short branch lengths relative to most other ferns, and were labelled "molecular living fossils" in keeping with their relative morphological stasis for the past 200 million years. We ascertain that tree ferns have indeed undergone dramatic decelerations in their rates of molecular evolution. Possible explanations are considered in the context of tree fern ecology and life history patterns.

P0897. Higher spore-bearing plants collection in outdoors in the O.V. Fomin Botanical Garden

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The collection of higher spore-bearing plants in outdoors was started in 1966 with a few common species. The exposition has been obtained by culture from spores and living plants from expeditions to the Far-East, Caucasus, Altai, Crimea, Carpathians, etc. The collection contains at present 120 taxa. There are 82 species, 6 subspecies, hybrids and varieties and 32 cultivars of ferns and horsetails. On the exposition plot grow the representatives of *Ophioglossaceae*, *Botrychiaceae*, *Osmundaceae* (sp. of genus *Osmunda*, *Osmundastrum*), *Onocleaceae* (sp. of genus *Onoclea*, *Matteuccia*), *Athyriaceae* (sp. of genus *Athyrium*, *Cornopteris*, *Cystopteris*, *Diplazium*, *Gymnocarpium*, etc.), *Blechnaceae*, *Dryopteridaceae* (sp. of genus *Cyrtomium*, *Dryopteris*, *Polystichum*), *Hypolepidaceae*, *Thelypteridaceae* (sp. of genus *Phegopteris*, *Thelypteris*, etc.), *Adiantaceae*, *Aspleniaceae* (sp. of genus *Asplenium*, *Phyllitis*), *Woodsiaceae*, *Polypodiaceae*. There are two rare species of Ukraine in collection *Asplenium adiantum-nigrum*, *Woodsia ilvensis*.

P0898. Diversity of Pteridophytes in China

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This video, introduces the diversity of pteridophyte in China, containing over 100 living species of ferns and fern allies. The first part demonstrates the sporophytes from NE to SW China. They are the representative of the Chinese pteridophytes, involving all the five subdivisions, i.e. Psilophytina, Lycophytina, Isoephytina, Sphenophytina and Filicophytina (Ching's system). The main body of this part deals with their form and structure. Geographically, there are not only the East Asia or Chinese endemic species, but also tropical and temperate members. Ecologically, various habitats are presented so as to understand why such a group of ancient plants is still flourishing in the world today. The second part records the diversity of the gametophytes of pteridophytes based on our own work. Like their sporophytes, they vary from each other in both adult prothallia and their whole course of development. Four typical patterns of gametophyte development, the *Osmunda* type, the *Adiantum* type, the *Ceratopteris* type and the *Aspidium* type, are pictured in detail. Spore morphology and apogamy, which also tell us the diversity of gametophytes, are given by tableaux.

P0899. The influence of soil evolution on pteridophyte diversification

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We combined ecological data with phylogenetic analyses to evaluate the potential role of soil differences on the diversification of pteridophyte lineages. We hypothesize that three crucial changes took place during the evolution of modern angiosperm forests: the development of a dark understory, of canopy habitats sites suitable for epiphytes, and of nutrient-rich soils fertilized by angiosperm leaf litter and decaying wood. Basal pteridophyte taxa, which appear to be obligatory mycorrhizal, evolved on nutrient-deficient raw soils, acidic soils derived from gymnosperm litter, or peat soils on water-logged sites. The evolution of angiosperms resulted in improvement of soil nutrient conditions, triggering several radiations of pteridophytes, especially among the modern ferns (polypod) that are facultatively mycorrhizal, but also among some basal lineages that managed to adapt to nutrient-rich soils. In contrast, basal lineages restricted to "ancient" soil types are currently species-poor.

P0900. New data of early leaf development of *Ophioglossum vulgatum* L. (Ophioglossaceae).

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The process of growing of the leaf and sheath from the apex of rhizome is the feature that can help in understanding of the phyllotaxy and even the taxonomy location of this species and its variations. We found out that the bud includes several (4-5) leaf primordium. Apex is situated lower than all the leaves. Every primordium is covered with its own sheath, and there is a cavity between them. Leaves are considered to grow, using cavities of the previous ones, but our data confutes this opinion. Cavities are not connected to each other, and all the leaves are embedded to the common tissue. Discover individual sheaths in this common tissue is quite difficult. New leaf doesn't grow to the cavity of the previous one and doesn't rupture the sheath of the previous leaf, but develops independently. Studying structure of three rhizomes on the transverse sections, we found no pores or holes between cavities there. Primordium-sheath complex develops, expanding and elongating, then growing of the sheath inhibits, and leaf perforates it. As a result, every petiole surrounded with a "collar".

P0901. Reticulate evolution of *Vandenboschia radicans* complex (Hymenophyllaceae) in Japan with special reference to its hybrid-dominant tendency

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Vandenboschia radicans complex is a filmy fern group distributed throughout the world, and was formerly considered as a cosmopolitan species. Plants of this complex are not rare in shady moist places of the Japanese Archipelago. Although four taxa are said to be present within quite a wide range of its morphological variation, boundaries between them are in fact ambiguous. Our study combining ploidy, CpDNA (*rbcl*) and nrDNA (*GapCp*) analyses has clarified that it is a hybrid complex containing diploid, triploid and tetraploid originated from complicated hybridizations and polyploidizations involving at least three parental diploid species. Sterile triploids show much wider distribution than fertile diploids/tetraploids, and grow in localities where no fertile individuals are found. Nevertheless genetic variation of triploids suggests they are of recurrent origins (i.e. they are never asexually reproduced clones). We discuss the reproductive system of these hybrid triploids, including the possibility of the involvement of independent gametophytes in it.

P0902. The study of morphology, sporology and anatomy of different species of the genus: *Polystichum* along with introducing the new species: *Polystichum setiferum* in Iran

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The genus *Polystichum* is one of the species of *Dryopteridaceae*. Distribution of this genus in Iran is mainly in humid woodlands regions of Caspian Sea coasts in Hyrcanian phytogeographical region and sometimes within rock slots in timberline.

Wendelbo(1976) introduced three species of this genus named *Polystichum braunii* (Spencer) Fee., *Polystichum aculeatum* (L.) Roth and *Polystichum woronowii* Fomin in Mon., which the last is classified a Hyrcanian plant.

In the current study on the ferns of Northern Iran, the species *Polystichum setiferum* (Forsk.) Woynar, is introducing in Iran for the first time.

However, the anatomy, sporology, morphology and sporangium of various species of this genus in Iran are studied; distribution map and determination key are provided.

P0903. A new checklist of north Iran ferns

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In a new study on the north Iran ferns, conducted in the hyrcanian phytogeographic region during the years 2001 to 2003, a total of 3 orders, 14 families, 22 genera and 40 species have been reported. In comparison to the reports of Wendelbo (1976), Denk (1998) and Jenkins (1980) which reported a total of 32 species for north Iran, this study has reported 8 new species, four subspecies and one variety in the Iranian flora for the first time.

The new species include:

1. *Asplenium onopteris* L.
2. *Athyrium distentifolium* Tausch ex Opiz.

3. *Dryopteris caucasica* (A. Braun) Frazer-Jenkins & Jermy
4. *Dryopteris carthusiana* (Vill.) H. P. Fuchs
5. *Dryopteris dilatata* (Hoffm.) A. Gray
6. *Dryopteris expansa* (C. Presl.) Frazer-Jenkins & Jermy
7. *Dryopteris oreades* Fomin
8. *Polystichum setiferum* (Forsk.) Woynar

The subspecies include:

1. *Dryopteris affinis* sub sp. *Affinis*
2. *Dryopteris affinis* sub sp. *Borreri*
3. *Asplenium trichomanes* sub sp. *Trichomanes*
4. *Asplenium trichomanes* sub sp. *Pachyrachis*

And the new variety is *Thelypteris palustris* var. *thelypteroides*.

P0904. Introducing new species of genus: *Dryopteris* along with morphology, sporology and anatomical studies and proposition of its determination key and distribution map in Iran

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The genus *Dryopteris* belongs to *Dryopteridaceae* family. This genus have a great morphological diversity in Iran and its distribution is mainly in humid woodland regions from low altitude of Caspian Sea coasts to timberline in Hyrcanian phytogeographical region.

Wendelbo(1976) introduced three species: *D. filix-mas* (L.) SCHOTT, *D. borrieri* NEWMAN and *D. pallida* (Bory) FOMIN for Iran flora.

Jenkins(1980) introduced *D. affinis* along with two subsp. named *D. affinis* subsp. *coriacea* and *D. affinis* subsp. *persica*.

In current study on ferns of Northern Iran, five new species of this genus named *D. caucasica* (A. Braun) Frazer-Jenkins & Jermy, *D. carthusiana* (Vill.) H. P., *D. expansa* (C. Presl.) Fraser Jenkins & Jermy, *D. dilatata* (Hoffm.) A. Gray and *D. oreades* Fomin. Have introduced for Iran flora.

Finally, along with anatomy, sporology and morphological studies, the determination key and distribution map of these species are provided.

P0905. Spore Morphology of the Caucasus Fern Species

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Ferns are rather important component of the Caucasus flora, especially, in Colchic and Tالش refuge. Pteridophyta of the Caucasus presents 70 taxon, that belong to 31 genera. 25 species are common with the North American ferns. New data on spore morphology for 65 species (47 monoolete-trilete/18) are given. The measurements of spores were made on the acetolyzed material, perisporium sculpture by SEM. Descriptions and microphotographs are presented. 7 main spore types are revealed. The data provides information for certain species, such as critical taxons of genera (*Asplenium*, *Cystopteris*, *Dryopteris*, *Polystichum*, *Woodsia*). The structural peculiarities of the spores, gives us possibility to discuss available relationship between taxons and ploidy level.

The altitudinal distributions of each species and its habitat requirements, possible relationships are discussed.

P0906. Molecular phylogeny of *Danaea* Sm. (Marattiaceae).

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The taxonomy and ecology of the neotropical fern genus *Danaea* (Marattiaceae) have been mostly neglected. However, an ongoing revision of *Danaea* for the Flora of the Guianas, Flora of Bolivia, and Flora Neotropica has led to the description of several new species. In turn, recent field studies have yielded an extensive collection of herbarium and silica-gel specimens of all species of *Danaea*. Here, we present the first phylogenetic estimate of relationships within *Danaea* based on a broad sampling of 70 specimens from various ecological and geographical sites using DNA sequence data from three plastid regions (*atpB*, *rbcl* and *trnL-F*). Taxa from *Angiopteris*, *Archangiopteris*, *Christensenia*, and *Marattia* are used to root the tree. We identify three major clades within *Danaea* corresponding to rhizome symmetry: one clade, consisting of all species with erect rhizomes, is sister to the other two clades where all taxa have creeping rhizomes. This phylogeny provides us with an explicit framework for addressing questions related to the morphological, ecological, and

biogeographical evolution of these ferns, all of which are crucial to a modern revision.

P0907. Molecular phylogeny of the Ophioglossaceae inferred from *rbcl* sequences with special emphasis on the genus *Mankyua*

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Ophioglossaceae comprises four genera including *Botrychium* s.l., *Helminthostachys*, *Ophioglossum* s.l., and *Mankyua* which was recently described from Jeju Island, Korea. The *rbcl* sequences from 31 species of Ophioglossaceae were analyzed using maximum parsimony analysis. Parsimony analysis resulted in eight equally parsimonious trees, which differed mainly in terminal relationship within *Ophioglossum* group. The trees obtained have a length of 811 steps with consistency index of 0.5338 and retention index of 0.8225. In the consensus tree, the monophyly of the family Ophioglossaceae was well supported. In addition, the tree suggested the presences of two major clades within the family; they represent the Ophioglossoid and Botrychioid lineages. The former lineage corresponds to the genus *Ophioglossum* s.l., and the latter lineage includes the remaining three genera. Within the Botrychioid lineage, *Helminthostachys* and *Mankyua* appeared to be sister to the *Botrychium* s.l., but the relationship was not strongly supported.

P0908. Morphological and chromosomal variation of the *Dryopteris varia* complex (Dryopteridaceae) in Korea

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Dryopteris Adans. is a highly variable genus including more than 200 species worldwide. We have examined the variation in morphology, chromosome numbers and reproduction modes among Korean populations of the *D. varia* complex. Numerical analyses (landmark analysis and principal components analysis) revealed seven entities within the *D. varia* complex in Korea; these comprise *D. varia*, *D. pacifica*, *D. sacrosancta*, *D. bissetiana*, *D. saxifraga*, *D. saxifraga-varia*, and the Suak population which is considered to be a new taxon. Mitotic chromosome counts and examination of reproduction modes indicated that *D. bissetiana* in Korea appears to be agamosporous with diploid or triploid chromosome numbers. However, *D. saxifraga* is sexual diploid or agamosporous triploid, and the other taxa are agamosporous triploid. *Dryopteris bissetiana*, *D. saxifraga* and *D. saxifraga-varia* are similar in major morphological characteristics, but show differences in attachment and shape of rachis scales. The results also suggest that agamosporous triploid *D. saxifraga-varia* was probably derived from hybridization between sexual diploid *D. saxifraga* and *D. bissetiana*.

P0909. When and where *Polystichum* s.l. originated? Evidences from molecular data

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Polystichum Roth is a nearly worldwide genus with its diversity center in southwest China and adjacent regions. In this study, 60 *rbcl* sequences of *Polystichum* species, including 23 new sequences from southwest China, were used to assess the phylogenetic relationships within the genus; on the basis of estimated evolution rate of *rbcl* and the relative-rate tests results, the divergence time between major clades were further estimated. The phylogenetic results reveal that all species of *Polystichum* s.l. in this study (including *Cyrtomium* and *Cyrtomidictyum*) form a monophyletic group. The basal split in *Polystichum* s.l. separates a clade with all Asian members from a clade containing other species from all over the world. The divergence time estimation results suggest that *Polystichum* s.l. originated in Asia in the late Late Cretaceous (176 Ma) and migrated into other places in the world in early Eocene (146 Ma).

P0910. Phylogenetic Relationships of the Neotropical Species of *Polypodium* (Polypodiaceae) based on Morphology evidence

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In this study a list of 65 characters and character states were used to fulfill a cladistic analysis of 35 *Polypodium* species based on morphological, anatomical and spore characters using PAUP version 3.1. These characters were unordered and unweighted. The sequence of random addition was done using MULPARS. The polarization of the characters was assessed using the standard procedure of outgroup comparison with *Polypodium californicum*. The analysis obtained produced 192 cladograms with a consistency index of 0.52, a homoplasy index of 0.062 and a retention index of 0.683. All the cladograms agreed on the following points: to reorganize the several groups of the genus, therefore to realize that we are naming *P. colpodes* group with 5 species, *P. plesiosorum* group with just 3 species, *P. dulce* group with *P. martensii* and relatives and *P. fraterum* group with *P. echinolepis* and relatives. We indicate the taxonomic affinity of *P. fallax*, *P. feuillei*, *P. kunzeanum*, *P. pleurosorum* and *P. rhachipterygium*. As well as we suggested the redefinition of *Pleopeltis* and *Phlebodium* genus.

P0911. Phylogenetic relationships in Pteridaceae inferred from chloroplast *rbcl* nucleotide sequences

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Pteridaceae are ferns distributed worldwide, with most genera centered in the tropics, usually with adaptations to xeric habitats. Most fern phylogenetic studies have relied upon *rbcl* sequences. In the present investigation *rbcl* sequences of Pteridaceae species native in Brazil and representative of *Acrostichum*, *Adiantopsis*, *Adiantum*, *Cheilanthes*, *Doryopteris*, *Eriosorus*, *Hemionitis*, *Pellaea*, *Pityrogramma*, *Pteris* and *Vittaria* (Vittariaceae) were obtained. These sequences and GenBank *rbcl* data from representatives of *Anogramma*, *Ceratopteris*, *Coniogramma*, *Jamesonia*, *Microlepia* (Dennstaedtiaceae), *Notholaena*, *Platyzoma*, *Taenitis* and *Trachypteris* were analyzed using parsimony and *Microlepia strigosa* as outgroup. The results indicate that Pteridaceae *sensu* Tryon et al. is paraphyletic, Pteridoideae, Cheilanthoideae *sensu* Tryon et al. and *Pteris* are polyphyletic. Adiantoideae, Ceratopteridoideae and Taenitidoideae are monophyletic. *Pteris cretica* from the Old World is sister to most neotropical *Pteris* species, while *Pteris vittata* clades with *Platyzoma*. *Acrostichum* (Pteridoideae) clades with *Ceratopteris* (Ceratopteridoideae).

P0912. A systematic study of the fern genus *Polystichum* (Dryopteridaceae) in Japan

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Hybridization and polyploidization play the important roles in species diversification and reticulation in the fern genus *Polystichum*. In this study, morphological, cytological and electrophoretic investigations were undertaken in eleven Japanese *Polystichum* species. The polyploidy and genetic patterns of four diploid species and seven tetraploid species were determined. The spore morphology showed the distinct characters in each diploid species, but intermediate among tetraploid species. Electrophoretically, the species-specific marker alleles were detected in four loci of PGM, PGI, SkDH, LAP enzymes in each of four diploid species *P. fibrilloso-paleaceum*, *P. igaense*, *P. retrosopaleaceum*, *P. otomasui*. Electrophoretic variants were detected in the tetraploid species *P. longifrons*, *P. tagawanum* and *P. polyblepharum* (all of them were allopolyploids). Further, six triploid hybrids and five tetraploid hybrids were examined morphologically and electrophoretically, and the putative parents of these hybrids were confirmed. Based on the above, the reticulate evolution of the Japanese *Polystichum* is discussed.

P0913. Phylogenetic studies in the genus *Doryopteris* J. Sm. (Cheilantheoideae-Pteridaceae)J. C. Yesilyurt^{1,2};¹Royal Botanic Gardens, Kew, Surrey, United Kingdom, ²The Natural History Museum, London, United Kingdom.

Doryopteris is the fourth largest genus of Cheilantheoideae-Pteridaceae. A number of species appeared to be congeneric mainly on the basis of soral condition and some of them proved difficult to classify within the genus or in related genera (*Cheilanthes*, *Pellaea* and *Notholaena*). The research was carried out in order to elucidate interspecific relationships within *Doryopteris* and to formulate evolutionary hypothesis of its relationships with the related genera. Data from cpDNA sequences (*trnL-F* and *rbcL*) and morphology were analysed independently and in combination using maximum parsimony. *Doryopteris* is paraphyletic according to the molecular data and polyphyletic with the combined analysis. However, the topologies are congruent with the two sections recognised by Tryon. Cytological data indicate that hybridisation and polyploidy are also playing a role in the evolution of this genus.

P0914. The study of morphology, sporology and anatomy of the species of the genus: *Athyrium*, along with introducing the new species: *Athyrium distentifolium* in Iran

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The genus *Athyrium* belongs to *Athyriaceae*. Distribution of this genus in Iran is mainly in humid woodlands regions of Caspian Sea coasts in Hyrcanian phytogeographical region and sometimes within rock slots in timberline.

Wendelbo(1976) introduced a species of this genus named *Athyrium filix-femina*.

In current study on the ferns of Northern Iran, the species *Athyrium distentifolia* Tausch ex., is introducing in Iran for the first time.

Noting to morphology, anatomy, sporology - and partly ecological - characteristics, could have been differentiated this species with its neighboring species.

The species *A. distentifolium* contains orbicular sori, without indosium - or soon lost - and spinulose spores. In transverse sectioning, petiole is siphonostele with six meristele.

The anatomy, sporology and morphology of this genus in Iran are studied and distribution map and determination key for its species are provided.

P0915. Palinology of twenty five species of the genus *Diplazium* (Woodsiaceae).

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The palinology of *Diplazium* species is studied as a contribution to the knowledge of the genus to ascertain its utility for the delimitation of close species and study the possibility to segregate groups or subgenus of *Diplazium*. Some of the spores were collected in the field, some others were taken from herbarium sheets. The spores were set on a coal tape and this one on a small aluminum stick. Some of the spores were broken to see the spore structure, later on they were covered with coal dust and gold. The micrographs were taken using a Scanning Jeol 35CF microscope. *Diplazium* spores are monolete, 25-75 µm, with a linear aperture 2/3 to 3/4 their length. The aperture, however, is often obscured by broad perispore wings. In most species these wings have irregular margins, but in others are almost entire to fimbriate. Sometimes, the perispore wings make a wide reticulate, in other times are irregular or make a cristate pattern. The exospore is plain. The perispore usually is of two layers, the lower of which has small papillae, whereas the upper layer is thinner and fragile. The elements between the folds are part of the perispore and they are variable between species.

P0916. Anatomy of Thirty Species of *Diplazium* (Woodsiaceae)

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The anatomical studies of thirty Neotropical species of *Diplazium* are providing to contribute to the knowledge of the genus and to ascertain its utility for the delimitation of species. Roots of all the

species are diarch, with a dictyostelic rhizome, a petiole with three possible xylem strands shapes: hippocampus, elliptical or walking sticks; in the distal petiole or in the rachis the strands are united in a single strand with rounded U or squared U shape. There are mucilage cavities in the xylem parenchyma of the leaf trace. The cortex of the root, rhizome and stipe has starch grains; cells with dense brown content are disposed near or around the meristemes, leaf traces, veins or medulla are present in almost all the species studied. In general the data show that the species present similar anatomy, each one can be differentiated from the others by its shape of the leaf trace, number of caulinar meristemes and distribution of brown content cells. The conclusions confirm the usefulness of anatomical and histochemical characters to delimitate species.

P0917. To a question on systematization *Ducampopinus krempfii* (Lecomte) A. chev. (Pinaceae)

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The structure of bark *Ducampopinus krempfii* till now remained unexplored. We carried out studying anatomy of young branches of this plant. Investigated by us taxon finds out similarity to *Pinus*, *Keteleeria* and *Pseudolarix*, however on the majority of attributes of a structure of a bark is closer only to first two. Third rather strongly differs on a lot of attributes: the form of cells of epidermis - ellipse; the form of cells of hypodermis; presence in an initial bark sclereids; under the form of cells of phellem in periderm-rectangular or square and some to other attributes. At the same time, at both genus's in a bark is absent resiniferous system and dominate numerous slime idioblastes, quantity crystal parenchyma insignificantly and is absent typical aerenchyma that specifies doubtless related affinity of these two genus's. Most precise difference *Ducampopinus* from relatives to it of sorts *Pinus* and *Keteleeria* is absence in an initial bark resin ducts and homogeneous phellem of periderm. Results of our anatomic research *Ducampopinus* represents close, but independent taxon in a rank of independent genus *Ducampopinus*.

P0918. Were Mesozoic Ginkgophytes Shrubby?

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Data on leaf morphology in the Mesozoic of North America shows a proportional increase of bifurcated, Ginkgo-like leaves during the middle of the Jurassic. This ginkgophyte acme is correlated with a decreased proportion of the leaf forms associated with herbaceous or shrubby pteridophytes, and with no substantial change in the proportion of leaf forms associated with canopy gymnosperms. The increase in Ginkgo-like foliage at the same time as fern-like forms decreased in relative abundance suggests replacement of some part of the forest understory by early ginkgophytes. That is, early ginkgophytes were competing for space in the understory rather than in the canopy. This data suggests that most Mesozoic ginkgophytes were not canopy trees like the surviving Ginkgo biloba, but shrubs, and may support the argument that has already been made from sedimentological data, that to a much greater extent than do individuals of Ginkgo biloba now cultivated around the world, many ancestral ginkgophytes pursued ruderal or early-successional strategies.

P0919. Young crown groups in *Gnetum* and *Ephedra* - reconciling molecular age estimates and fossils in GnetalesH. Won^{1,2}, S. S. Renner³;¹University of Missouri-St. Louis, Department of Biology, St. Louis, MO, United States, ²Seoul National University, School of Biological Sciences, Seoul, Republic of Korea, ³Ludwig Maximilians University Munich, Munich, Germany.

Gnetales comprise *Gnetum* (35 spp.), *Welwitschia* (1 sp.), and *Ephedra* (35+ spp.). An *rbcL* clock gave an age of 32-8 my for the initial divergence among living species of *Ephedra* (Huang & Price 2003), yet fossils with modern reproductive characters existed 110 mya (Rydin et al. 2004; Yang et al. 2005). Using slow-evolving *rbcL* and fast-evolving *matK* for dense species samples, we estimated the ages of the initial divergences in *Ephedra* and *Gnetum*. Both genes, regardless of seed plant topology and clock approach used, yield basal divergences in *Gnetum* of 22-14 my and in *Ephedra* of 16-2 my. The ca. 35 species in each genus appear the result of recent radiations from ancestors that kept

certain morphological traits (unknown for *Gnetum*), while congeners went extinct. This seems as plausible as extant species being of Mesozoic age (Rydin et al. 2004). If true, the ages of the *Gnetum* clades (S American (African, Asian)) imply that the genus reached its distribution across water (n.b., some *Gnetum* have buoyant seeds). Recent speciation, esp. in Asia would explain the limited morphological divergence, overlapping distribution ranges, and incomplete concerted evolution in rITS.

P0920. Phylogeny within Cupressus L. with morphological data

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Recent studies on the phylogeny of Cupressus L. are mostly based on molecular data. Even if morphological data are considered then they are often incomplete and sometimes even not correct, because they are based on outdated information. Moreover, none of these trees includes all species of the genus. The understanding of evolution requires careful morphological interpretation of such phylogenetic trees. For the present study, a cladogram was calculated including each Cupressus species (incl. Xanthocyparis FARJON & HIEP) for the first time. This cladogram is based on a matrix of characters resulting from new morphological investigations and critical literature analysis as well. The topology resulting from this study fits well to the spatial patterns of the species. The two species of Xanthocyparis (Xanthocyparis vietnamensis FARJON & HIEP, Xanthocyparis nootkatensis (D. DON) FARJON & HARDER syn. Chamaecyparis nootkatensis (D. DON) SPACH) appear in different positions within Cupressus, therefore the genus has to be included in Cupressus.

P0921. Jurassic Araucaria from the sauropod dinosaur bonebed at Howe Ranch, Wyoming: the power food of the giants?

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Nearly all specimens of a new flora from a dinosaur bonebed (G Quarry, Howe Ranch, Wyoming) in the Late Jurassic Morrison Formation pertain to various plant organs of *Araucaria*. Large, squat cones with partially fused ovuliferous scale-bract complexes which are shed at maturity represent the ovulate strobili. The ligulate, unwinged, single-seeded nature of the cone-scale complexes and the detached unwinged seeds show similarities to the extant species *A. bidwillii*, Section *Bunya*. Slender cones with long-bristled cone scales are likely microstrobili. Branches and twigs bearing rhomboidal, scale-like leaves of the form genus *Brachyphyllum* represent araucarian foliage and hint at locally arid conditions. Coniferous wood is also present in large quantities. Fermentation experiments with material from extant *Equisetum*, ferns, tree ferns, conifers, and selected basal angiosperms indicate that modern leaves of *Araucarias* spp. have a comparatively high energy content and nutritional value for browsing herbivores. Virtually the only fossil plant at the site, *Araucaria* may have played a major—if not exclusive—role in the diet of these gigantic sauropods.

P0922. Genetic variation of Ginkgo biloba based on RAPD, ISSR and PCR-RFLPs

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Ginkgo biloba is referred as a "living fossil" plant. As a wild species, it is native to China. Patterns of cpDNA variation were studied in 220 individuals from 15 populations inside and outside China. Six populations in China were selected to analyze population differentiation using ISSR and RAPD markers. Results show $PPB = 73.86\%$ in ISSR and $PPB = 68.04\%$ in RAPD. Combined data indicated relatively high $H_e = 0.2408$, $H_o = 0.3599$ in species level. Possible natural populations possessed higher genetic diversity. Differentiation among populations is 28%. Restriction analysis of PCR-cpDNA fragments was employed to identify the distribution of haplotypes. There were 19 haplotypes in two combinations. Haplotype A was the most frequent haplotype, found in all populations. Haplotype B, G and H represented 11.4%,

16% and 5% of the total samples. Other 15 haplotypes were very rare, but some of them were informative. Distribution of haplotypes revealed diversity center lies in Guizhou and Congqing of Southern China, possible natural populations. Combined ecological and genetic data, Two conservative strategies are proposed.

P0923. Fine morphology of peculiar reticulate pollen from the Permian of Russia

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The morphology of dispersed pollen of Reticulatina from the Permian of the Russian Platform was studied. We found individual pollen grains showing reticulate pattern both under LM and SEM (group 1) and a different set of specimens (group 2) with the inner reticulum visible only under LM. With SEM, group 1 exhibits a coarser reticulum in the center of the pollen grain and a finer peripheral reticulum. The ectexine includes perforated tectum, spongy infratectum with rather regular short partitions, and foot layer. The thick endexine appears nearly homogeneous, but at places lamellate structures are distinguishable supporting that the endexine might have been ontogenetically lamellate. Group 2 shows a continuous and smooth tectum that completely hides the underlying layers when examined with SEM. Differing in the ultrastructure of ectexine, they are similar to group 1 in the endexine morphology. Although the pollen grains are of gymnospermous affinity, the similarity between the surface of Reticulatina (group 1) exine and that of Cretaceous angiosperm pollen is fascinating. The study is supported by the RFBR, no. 03-04-49611.

P0924. Morphology and development of seeds of Zamia amblyphyllidia D.W.Stev. (Cycadaceae) and Ginkgo biloba L. (Ginkgoaceae)

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Our investigations about the female reproductive structures of *Zamia amblyphyllidia* D.W.Stev. and *Ginkgo biloba* L. were focused on the initiation and the development of seeds. Studies were done by SEM and LM to compare the results with previous studies in other gymnosperms. These results show various similarities within all members of Cycads and *Ginkgo*, but several differences to the morphogenesis of seeds in most Conifers. The differentiation of the integument in a stony sclerotesta and a fleshy sarcotesta in *Ginkgo* and Cycads has been mostly regarded as a derived feature. A detailed comparison of these two taxa should show whether this resemblance is a homology or not, and if it has to be regarded as a plesiomorphic or an apomorphic character within gymnosperms. It is apparent that the sarcotesta in both Cycads and *Ginkgo* is not homologous to the sarcotesta in *Cephalotaxus*. The latter appears to be homologous to the aril in other Taxaceae.

P0925. A comparative study of the sarcotesta of Cephalotaxus Sieb. & Zucc ex Endl. and the aril of Taxaceae

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The outer fleshy layer of the seed of *Cephalotaxus* was mostly regarded to be a sarcotesta and therefore as a structure completely different from the aril of Taxaceae. Therefore, the monogeneric family of Cephalotaxaceae was placed within the classical conifers distinct from Taxaceae, which was placed in their own order Taxales. In contrast to this view, recent molecular analyses group the Cephalotaxaceae and the Taxaceae as sister groups within classical conifers. For a better understanding of these groups, we have studied the morphogenesis, the morphology and the anatomy of the female reproductive structures of both groups by SEM and light microscopy to search for morphological homologies which support the high affinity of both families in molecular analyses.

We found that the sarcotesta of *Cephalotaxus* exhibit a striking similarity to the aril of *Taxus* L. and *Torreya* Arn., while the sarcotesta of *Zamia* and *Ginkgo* differs markedly from the fleshy layer of *Cephalotaxus*. Therefore, the clustered position of *Cephalotaxus* and the rest of Taxaceae is also supported by morphological characters and *Cephalotaxus* might be best included in Taxaceae.

P0926. Early lignophyte trees

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Early evolution of the tree habit in the lignophyte clade is poorly understood and patterns of diversification of the trees that replaced the progymnosperm genus *Archaeopteris* around the Devonian/Carboniferous boundary remain obscure. We focussed our research on woody axes from the marine deposits of Thuringia (Germany) and Montagne Noire (France) dating from the basal Mississippian (Tn2b-Tn3a). Such deposits are known to yield plant remains from a wide range of environments from lowlands to well-drained elevated areas. The axes show a broad diversity of stelar and wood anatomy and some represent new taxa. Genera previously described from younger deposits of northwestern Europe (i.e. *Protopytis*, *Eristophyton*) are also present, which widens their recorded occurrence stratigraphically to the basalmost Carboniferous, and geographically to the northwestern border of Gondwana. These results shed more light on the diversity of arborescent lignophytes soon after the D/C boundary, suggesting that they originated in the late Devonian while *Archaeopteris* was still flourishing.

P0927. On the systematic importance of the morphology and anatomy of the vegetative and reproductive organs in the Pinaceae

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Studies of different genera of Pinaceae have shown that various (partly new) morphological and anatomical characters (1) of both vegetative and reproductive organs are of great systematic value. In Pinus, these 1 include needles (their dimensions, number per brachyblast (2), shape (3) in cross-section and apex, degree of marginal dentation), sheaths of 2 and scale-like leaves (3 and color, arrangement on axis, morphology (4) of marginal fringes etc.), prophylls of 2 (sizes and 3, 4 of their carina), apical buds (dimensions and 3) and their scales (4 of marginal fringes), young shoots (thickness, hairiness, color etc.), microstrobili and microsporophylls (size and 3 of clusters of microstrobili and of each microstrobilus, 4 of supra-connective and connective), cones and their scales. Based on dehiscence type of paired microsporangia, genera of Pinaceae are referable to 3 groups: - a) dehiscence along transverse suture (*Abies*, *Keteleeria*, *Tsuga*, *Pseudolarix*), - b) along longitudinal-medial (*Larix*), and - c) along longitudinal ones (*Pinus*, *Cedrus*, *Pseudotsuga*, *Picea*). Within Pinaceae Pine species have the most specialized microsporophyll structure.

P0928. AFLP Phylogeny versus fossil evidences within genus Araucaria

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The genus *Araucaria* (Araucariaceae) includes four sections (19 extant species) restricted to the Southern hemisphere. The fossil register indicates that this genus was widespread also in the Northern hemisphere and denotes *Eutacta* and *Bunya* as the oldest sections. About 670 polymorphic AFLP markers were applied to analyse the phylogenetic relationships of seven species from three sections within genus *Araucaria* with a cladistic (parsimony) and a phenetic (NJ with Nei and Li genetic distance) approaches. Phylogenetic trees obtained from AFLP data are collinear with a *rbcl* tree and support the classification in sections according to morphological characters. Sections *Eutacta*, *Araucaria* and *Bunya* are supported as monophyletic groups by high bootstrap values in the AFLP analysis. In accordance with the fossil record section *Eutacta* has a basal position within genus *Araucaria*. However, section *Bunya* diverged latter and is sister to section *Araucaria*.

P0929. Cone and seed structure of Thuja sutchuenensis

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Morphology and anatomy of cones and seeds of *Thuja sutchuenensis* (critically endangered endemic of south-west China) were studied in compare with other species of the genus. Mature cone is brownish, up to 1 cm; cone scales (8-10; 4-6

medial are fertile) up to 6.5 mm with 1-2 axillar seeds. Seed body is ellipsoidal lightly brown, up to 5 mm, with two equal lateral crescent-shaped wings. Seed coat is formed by exotesta (typical epidermis of cells with tannins in walls and pale lumens), mesotesta with resin ducts (12-13 layers of pale isodiametric cells with thickened unligificated walls) and endotesta (1 layer of cells with thickened unligificated walls and tannins in lumens). The structure of female fructifications of *Th. sutchuenensis* implies basal position of the species in the genus. Other *Thuja* species are characterized by more advance features of female fructifications, especially in the structure of seed coat (e.g. reduced number of cell layers of mesotesta). Supposedly, *Th. sutchuenensis* inhabits the ancient refugium of the genus, from where other representatives of *Thuja* distributed.

P0930. The genofund of conifers in Azerbaijan and the questions of its diagnostics.

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The conifers in Azerbaijan are represented with ancient relict and endemic taxons, which are similar but always complemented by scientific material. The high stability in different ecological conditions and conservatism of the conifers structures appeared during the evolution process. The paleobotanical researches conducted in Azerbaijan demonstrate that in the past geological epochs conifers took the dominant location. Our researches showed, in order to establish species characteristic of conifers, it is necessary to collate complex diagnostic elements including paleobotanical and phylogenetical data. Some ancient species (*Taxus*) require ultrastructural and morphophysiological researches as well as the diagnostical data. Based on the complex researches of the conifers of Azerbaijan the 3 species of *Pinus*, 7 species of *Juniperus* and 2 species of *Taxus* with new described species for science (*T. talyschensis* Gum.) have been established. The population of *T. talyschensis* is located on the upper border of the wood in Talish and there are about 100 of them. Their age is 1200-1400 years and they are in the process of degradation.

P0931. Assembling the tree of life for angiosperms

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Angiosperms are the largest and economically most important group of green plants. Despite enormous progress, several major nodes remain unresolved. The Angiosperm ATOL (Assembling the Tree of Life) consortium will attempt to clarify relationships at 12 problematic nodes. Targeted sequencing of 17 genes (~25,200 nt), representing the three plant genomes, for 370 species will provide a backbone tree, and entire plastid genome sequences for 30 angiosperms will be combined with published data to resolve relationships among major clades. Four large clades_Malpighiales, Ericales, Lamiales, and campanulids_will be examined intensively. We will compare supertrees with trees obtained through analyses of supermatrices. A "Botany Browser" will serve as a portal to an informatics infrastructure that will bring varied data resources together on a user's desktop. The Angiosperm MorphBank will archive annotated images of morphological features and will facilitate the diagnosis of clades, analyses of character evolution, and integration of fossils. This project will lay the groundwork for collaboratively building the tree of all 12,000 genera of angiosperms.

P0932. Cytotaxonomic congruence with five major clades in the Resedaceae based on ITS sequences.

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The Resedaceae (6 genera and c. 70 species) is widely distributed in the Old World, with some species in the New and a major center of diversity in the Mediterranean basin. Phylogenetic analysis of 62 ITS sequences reveal that *Reseda* (c. 60 species) is not a monophyletic group since two of the five major clades of Resedaceae include species of *Reseda* together with those of *Randonia* and *Oligomeris*. Two independent clades are in turn formed by *Sesamoides* and *Caylusea*. Three basic chromosome numbers ($x = 5, 6, 7$) and morphological characters served historically to circumscribe five sections in *Reseda*, taxonomic treatment mostly in agreement with the ITS phylogeny. Naturalness of smaller groups in *Reseda* implies: (1) monophyly of

most conspecific populations; (2) three endemics to different Iberian mountain ranges (sect. *Glaucoreseda*). At least three independent dispersal events may have been involved in *Reseda* colonization and differentiation in the Canary Islands: *R. luteola* (Old World), *R. lancerotae* (endemic), and *R. scoparia* (endemic).

P0933. Revision of *Butia* (Becc.) Becc. (Butiinae, Cocoeae, Palmae)

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Butia (Becc.) Becc. (Butiinae, Cocoeae, Palmae) is a neotropical genus composed by small to medium-sized palms that can be distinguished within other genera of the subtribe Butiinae by its strongly arched leaf rachis, smooth to slightly striate peduncular bracts, which sometimes are densely tomentose, and for the endocarp pores located nearly to or slightly below its equator. *Butia* occurs mainly in the grasslands and open fields from Brazil to Argentina, Paraguay and Uruguay, a few of them reaching the Atlantic lowlands, in coastal forest and sand dunes. Eleven species are here recognized: *B. archeri*, *B. campicola*, *B. capitata*, *B. eriospatha*, *B. leiospatha*, *B. leptospatha*, *B. microspadix*, *B. odorata*, *B. paraguayensis*, *B. purpurascens* and *B. yatay*. Two new combinations and 4 synonyms are proposed. The study includes a taxonomic key to the species, illustrations and color plates of the habits, morphological details and leaflet anatomy of the species. On a conservational perspective, the majority of the species in the genus can be considered endangered, as they occur at places under strong pressure of land use interests

P0934. Asian origin, Mediterranean differentiation and back-colonization of ivies (*Hedera* L., Araliaceae)

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Placement of *Hedera* in molecular phylogenies, embedded in Asian lineages of the Araliaceae and the finding of the oldest *Hedera* macrofossil in Asia, supports the argument of Asia as the center of origin for ivies. The most relevant diversity center is in the Mediterranean, as revealed by morphology, ploidy variation -four ploidal levels in Mediterranean vs only diploids in Asia-, and chloroplast diversity -four chlorotypes (I, II, III, IV) in the Mediterranean vs one (III) in Asia. A comprehensive trnT-L chloroplast study, with special emphasis in Asian populations, confirms that the most ancient *Hedera* chlorotype (cpl) is confined to W Mediterranean basin; and the presence of a single, derived chlorotype (cplIII) in Asia. These results lead us to hypothesize a diploid (cpl) Asian *Hedera* colonizing the Mediterranean. Once established here, a profound morphological, cytogenetic and molecular diversification took place. In parallel an extinction of Asian ivies occurred. After this, diploid ivies with cplIII recolonized Asia producing a most limited diversification.

P0935. Plastid DNA phylogeny and the classification of palms

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During the last ten years a number of phylogenies have been published for the Arecaceae. We build upon these studies and include all genera of palms in a cladistic analysis of plastid DNA sequences. One species from each genus was included as placeholder and for most monogeneric tribes and subtribes several species were included. Ten species from the Commelinid clade of monocots were included as outgroups. Four plastid DNA regions were sequenced for all 220 species, *rbcL*, *trnL-trnF*, *rps16* intron, and *matK*. The parsimony analysis of the combined data sets resulted in a phylogeny with good resolution at the subfamily, tribal, and subtribal level. The results corroborated previous molecular phylogenies and resolved five subfamilies, Calamoideae, Nypoideae, Coryphoideae, Ceroxyloideae (including Phytelphantoideae), and Areoideae as monophyletic. Additionally, the tribes and subtribes within Calamoideae supported the classification of Baker et al.; the Coryphoideae and the Ceroxyloideae were well resolved at the tribal and subtribal level; leaving Areoideae well-resolved at the tribal level but needing additional attention at the subtribal level.

P0936. Is the Strait of Gibraltar an effective barrier to gene flow in the wind-pollinated species *Carex helodes* Link (Cyperaceae)?: a molecular and cytogenetic approach.

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Carex helodes Link, previously thought to be endemic to SW Iberian Peninsula, has been recently found in a recent field campaign in northern Morocco. Phylogenetic reconstructions based on ITS and *matK* sequences, including populations of *C. helodes* from both sides of the Strait of Gibraltar, reveal its monophyly and agree with its circumscription in sect. *Spirostachyae*. Distribution of genetic diversity estimated from results of an AFLPs analysis does not allow establishing two different evolutionary lineages (Iberian vs. Moroccan populations). However, one exclusive character has been detected for Moroccan populations in the analysis of the ITS sequences. Furthermore, from a cytogenetic point of view, Moroccan populations have stabilized a single chromosome number $2n=74$, whereas Iberian's most frequent cytotype is $2n=72$. This suggests that the current geographical distribution of *C. helodes* is the result of a relatively recent dispersal event between SW Iberian Peninsula and N Africa, but reproductive and genetic gene flow between both sides of the Strait of Gibraltar cannot be ruled out.

P0937. Molecular markers help clarify morphological-based taxonomic studies of the UK National Willow Collection

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The understanding of relationships between species of the genus *Salix* and their identification is key to efficient utilisation of willows as bioenergy crops. The current taxonomy of the genus *Salix* is very complex and is being continually revised.

The National Willow Collection (NWC) was originally established in 1920 as a repository for disappearing willows used for basketry. Now it is the only National Collection of *Salix* comprising more than 150 species and hybrids from all over the world. We are undertaking a thorough characterisation of the Collection using traditional botanical knowledge and modern molecular techniques to provide improved knowledge of germplasm available. Morphological assessment carried out on the NWC and herbarium material demonstrated that key characters used for the identification of some species were unreliable due to the large intra-specific variation, especially within species of the section *Vimen*. Amplified Fragment Length Polymorphism (AFLP) analysis carried out on DNA extracted from 64 species of *Salix* was successful in differentiating between species and supported known species relationships.

P0938. Evolution and Biogeography of Geraniaceae

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Geraniaceae is a monophyletic family included in the Geraniales (basal Rosids) together with four families (*Vivianiaceae*, *Francoaceae*, *Greyaceae*, and *Meliantaceae*). Geraniaceae s.l. consists of six genera (*Hypseocharis*, *Pelargonium*, *Monsonia*, *Erodium*, *Geranium* and *California*), which form a natural group with *Hypseocharis* sister group to the remaining genera. It has been proposed that an early Geraniaceae divergence occurred after the separation of South America and Africa. A limited distribution in central Andes and accumulation of primitive characters lead us to interpret a relict status of *Hypseocharis*.

A phylogenetic, biogeographic, and molecular study is herein presented to assess previous taxonomic treatments, to infer the historical distribution, to find out patterns of diversification, and to explore the rates of nucleotide substitution related to morphological differentiation. In this study we describe the patterns of genera diversification already described by Price & Palmer (1993) as well as a basal position of *California* in the Geraniaceae. We have also found one of the highest substitution rates in *rbcL* in angiosperms.

P0939. Systematics of *Pseudotrimezia* (Trimezieae, Iridaceae)

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Pseudotrimezia (Iridaceae) is an endemic genus from Espinhaço Range, Minas Gerais State, Brazil. It comprises about 20 species, with an interesting distribution pattern. The species, which are difficult to delimit, occurs isolated, sometimes to a single locality, being a good model for biogeographic studies. The genus has never been subject of phylogenetic analysis. Previous studies were made with *Pseudotrimezia* and related genus, *Trimezia* and *Neomarica* (tribe *Trimezieae*) by Rudall, using anatomical data, but not in a cladistic approach. The present preliminary phylogenetic analysis of *Pseudotrimezia* and tribe *Trimezieae* was based on morphological characters from literature, herbarium collections, and fresh material, as the collections of Iridaceae are almost always fragmentary. Differently from the previous analysis, the results show *Neomarica* as a clade within *Trimezia*, and *Pseudotrimezia* as a monophyletic group.

P0940. Revision of species of genus *Coccoloba* P.Brown nom. cons. (Polygonaceae) of Brazil

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A taxonomic revision of the genus *Coccoloba* from Brazil is presented. It is a Neotropical genus with about 400 species, distributed from the Southern United States (Florida) to Uruguay. The taxonomic treatment included nomenclatural revision, synonymizations, typifications, morphological descriptions, analysis of the geographical distribution patterns, comments and illustrations to Brazilian species. They were recognized 45 species; 18 new synonyms were proposed and 10 new occurrence of species from Brazil were recorded; 7 lectotypes and 2 neotypes are being designated here, and 2 species were re-established. This study includes an identification key, descriptions, illustrations, data of flowering and fruiting periods, general comments and analysis of distribution patterns of the taxa. Among the 45 species recorded at present, 20 occur exclusively in Brazil. In relation to geographical distribution patterns 16 species presented wide distribution; 19 species have restric distribution; 4 species present disjunct distribution pattern; and 6 species have an endemic distribution within Brazil.

P0941. Revision of *Aegiphila* Jacq.(Lamiaceae) and its sistematic position

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A revision of the Neotropical genus *Aegiphila* (Lamiaceae) is presented. Its taxonomic history is detailed and some morphological structures are re-evaluated. A palynological study is presented using both light and scanning electron microscopy. The geographical distribution is presented in ecoregions and the genus has 44 endemic species. The positioning of the genus at ordinal level as well as the positioning of the species in the genus are discussed using cladistic analysis based on morphological characters. The taxonomic revision resulted in the recognition of 102 species from the universe of 355 names previously cited for the genus. These species are classified in the two sections here recognized, whose delimitation, composition and monophily are defined and discussed. 74 new synonyms are proposed and one species is excluded from *Aegiphila*. The study of the nomenclatural types resulted in the designation of 49 new lectotypes, 8 epitypes and 3 neotypes. It is provided a dicotomic key for sections and species. For each species it is presented a list of synonyms, descriptions, distribution maps and discussion about its taxonomy and typification.

P0942. What-if anything-is Escalloniaceae?

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Escalloniaceae have traditionally been treated as a taxonomical dustbin. As a subfamily in Saxifragaceae sensu Engler 1928 it contained 16 genera, but have recently been slimmed down to only five genera (APG II), although their relationship with several of the genera formerly placed in Escalloniaceae are still uncertain. Using a broad sample with several representatives from all major campanulid clades, combined with Bayesian inference, a well-supported and monophyletic Escalloniaceae includes *Anopteris*, *Eremosyne* (formerly *Eremosynaceae*), *Escallonia*, *Forgesia*, *Polyosma* (formerly *Polyosmaceae*), *Tribeles* (formerly *Tribelaceae*), and *Valdivia*, but not *Quintinia*. This latter genus should together with *Sphenostemon* be transferred to

Paracryphiaceae. Furthermore, preliminary results indicates that *Valdivia* and *Forgesia* might be included in *Escallonia*. The age and biogeography of Escalloniaceae and its position within the campanulids is briefly discussed.

P0943. Morphological and Taxonomic Studies of the Genus *Tradescantia* L. (Commelinaceae) in Brazil

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This work reports a part of the studies about Brazilian Commelinaceae species. The study was based on a comparative morphological analysis of specimens obtained from herbaria and cultivated materials. Identification keys were made to species level. Descriptions and comments related to taxonomy, morphology, phenology and geographical distribution are included, as well as plates of flowering branches and floral morphology. Eight species were identified: *Tradescantia ambigua*, *T. anagallidea*, *T. blossfeldiana*, *T. cerinthoides*, *T. crassula*, *T. fluminensis*, *T. umbraculifera* and *T. zanonina*. *Tradescantia blossfeldiana* represents the first reference to Brazil. New occurrences to Brazilian regions are indicated: *T. ambigua* and *T. crassula* to Southeast, *T. umbraculifera* to South, *T. zanonina* to North, Northeast and South regions. The geographical distribution of *Tradescantia* species is more concentrated in the Southeast and South regions; however, *T. ambigua* occurs in the Northeast and Middle-West while *T. zanonina* has been found in the North and Northeast regions.

P0944. Morphological phylogeny of *Anthurium* Schott (Araceae)

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A partial cladistic analysis of *Anthurium* was conducted in order to define monophyletic groups and understand the evolution of morphological characters within the group. We studied 95 morphological characters in 30 species of *Anthurium* plus one species of five other genera of Araceae (i.e., *Asterostigma*, *Monstera*, *Philodendron*, *Pothos*, and *Spathicarpa*) that were used as outgroups. The species sampled represent a significant proportion of the morphological diversity of the genus and of the sections currently recognized in *Anthurium*. A parsimony analysis revealed two major groups within *Anthurium*, those of which were supported by a series of characters, including: the overall shape of the petiole and lamina, the shapes of the base and apex of the lamina, the number of cell layers in the palisade parenchyma, the position of the spathe, the sessile spadix, and berry color. On the other hand, important characters supporting minor clades included: the number and prominence of the 2ary veins and the number and presence of basal veins. Our results suggest that the currently recognized sections and series within the genus are not monophyletic. Cnpq, Fapesp.

P0945. Phylogenetic analysis of Fouquieriaceae based on morphological and molecular data

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Chloroplast DNA was obtained from ten taxons of *Fouquieria* (*F. burragei*, *F. columnaris*, *F. diguetii*, *F. fasciculata*, *F. ochoterena*, *F. purpusii*, *F. splendens*, *F. splendens* ssp. *breviflora* and *F. splendens* ssp. *splendens*) and one from outgroup (*Arbutus glandulosa*). Segments of 2,500 bp were amplified using trnKe1 and trnKe2 primers. PCR products were restricted with 8 endonucleases; and separated by horizontal electrophoresis, then documented for phylogenetic analysis; also morphological characters were documented to perform phylogenetic analysis. Analysis were performed using only the molecular data, or the morphological data, or both kind of data together (total evidence); methods of phylogenetic analysis were unweighted maximum parsimony and neighbor joining, with branch support tested with a 1000 resampling bootstrap. Phylogenetic trees obtained show a monophyletic origin for the Fouquieriaceae and just one genus: *Fouquieria*, which includes the woody and the fleshy species. *F. burragei* is a nearby species of *F. splendens*, so we can conclude that the division of the *Fouquieria* subgenus is not natural.

P0946. Modern approaches to the differentiation and taxonomy of genus *Anemone* L. (Ranunculaceae Juss.)

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In a re-examination of the evolution and taxonomy of *Anemone* L. (Ziman et al., 2004), we utilized several additional essential characters including chromosome numbers and pollen grain types, carpel morphology (sessile or stalked, styles distinct or indistinct, stigmas linear or dilated), stamens (filaments filiform or dilated), tepals (deciduous or persistent, monomorphic or dimorphic, venation including anastomoses), staminodes, germination, roots (taproots or adventitious), shoots above ground (mono- or sympodial, semi- or un-rossetteous) or underground (caudices, rhizomes or bulbs), basal leaves (petioles basally narrow, vaginated or sharply dilated, blades monomorphic or dimorphic, developing before or after anthesis) and involucre leaves (petiolate or sessile, similar or dissimilar to basal ones). As a result of the 150 species studied in this research, we are recognizing 4 subgenera, 23 sections, 4 subsections and 26 series, and we described subgenus *Stolonifera*, section *Rosulantes*, subsection *Somalienses*, and 14 series (*Nikoenses*, *Deltoideae*, *Trullifoliae* and others).

P0947. Phylogenetic relationships among *Gagea* and *Lloydia* (Liliaceae)

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As higher level relationships of the monocots are resolved, the need increases for comprehensive, lower level, generic and species studies employing rigorous phylogenetic and morphological character analyses. The systematics of *Gagea* and *Lloydia* species has long been considered difficult because only a few phenotypic features within both genera exist and as a result of hypothesized interspecific hybridisation; the latter we could show for *G. pomeranica* which represents a hybrid of *G. pratensis* and *G. lutea*. In the present study we report on the utility of intergenic regions in the plastid DNA (*trnL-trnF*) and of the internal transcribed spacer region of nuclear ribosomal DNA (ITS1, 5.8S rRNA, ITS2) to assess interspecific and generic relationships. Our cpDNA sequence data, the initial morphological analysis as well as the literature study, all indicate that the taxonomic position of *Lloydia* as an independent genus within the Liliaceae seems to be doubtful. However, a clear understanding of relationships among *Gagea* and *Lloydia* will require data from other DNA regions and additionally a more complete taxon sampling of both genera.

P0948. Ancestral types of minor vein phloem for subclasses of dicotyledonous plants

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Comparative study of the minor vein ultrastructure has been performed by TEM in leaves of 1500 species from evolutionary "advanced" and "primitive" (according to Takhtajan, 1997) families of dicots. Significant differences in organization of minor vein phloem have been revealed between evolutionary "young" and "ancient" families. Several characteristics delimit the "primitive" ancestral and the specialized types of minor vein phloem: (1) symmetry of companion cell-sieve element (CC-SE) complexes; (2) the number of CC-SE complexes per a minor vein; (3) the number of CC per SE; (4) the presence and size of plasmodesmal fields at the CC/bundle sheath interface; (5) type of plastids in CC. Only a small number of dicot families has specialized minor vein phloem, while their majority have no traits of minor vein specialization. We conclude that the ancestral field of non-specialized minor vein phloem comprises the basic taxa of several subclasses of dicots, and that specialized types have evolved several times in course of the evolution of dicots.

P0949. Morphological and molecular diversity in different *Asparagus* species (Asparagaceae) of Sri Lanka

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Morphological and rDNA ITS sequence studies were carried out to evaluate the number of different *Asparagus* that occur in Sri Lanka. Data were analysed independently and in combination and all analyses resulted in five distinct groups. Two of these groups unequivocally agreed with *A. gonocladus* Baker and *A. falcatus* L. *Asparagus* included under *A. racemosus* Willd. contributed to the remaining clusters and each could be supported by unique character combinations; *Asparagus* with shorter and sickle shaped cladodes with tapering base of higher elevation and lower elevation versus *Asparagus* with longer, straight cladodes of lower elevation. Worldwide Flora treatments for *A. racemosus* Willd. parallels well with shorter cladodes that occur in the low country. *Asparagus* that occur in the higher elevations is possibly *A. zeylanicus* (Baker) Hook. f. and needs to be resurrected. The study proclaims *Asparagus* described under *A. racemosus* Willd. is an assemblage of different species. Floral morphology for all these groups are under investigation. Further, fast evolving plastid regions will be sequenced construct phylogenetic relationships within this genus.

P0950. YABBY gene expressions in lateral organs of basal angiosperms

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Most YABBY genes (CRABS CLAW, FILAMENTOUS FLOWER, INNER NO OUTER, YABBY2, and YABBY3) are expressed on the abaxial side of lateral organs, such as leaves, and are involved in the establishment of abaxial tissue identity in core eudicots (Brassicaceae, Solanaceae, and Plantaginaceae). On the contrary, some YABBY genes are not expressed limitedly in the abaxial tissues of lateral organs in Poaceae. Here we report the expression patterns of YABBY genes in the lateral organs of the early branching angiosperms. We find that YABBY2-homologue of *Amborella trichopoda* (Amborellaceae) is expressed in the adaxial tissues of the carpel and leaf, in contrast to abaxial expression in core eudicots. In addition to this, YABBY5-homologue of *Cabomba caroliniana* (Cabombaceae) is also expressed in the adaxial tissues of the carpel. This difference indicates the abaxially-limited expression pattern of the YABBY genes evolved in the course of angiosperm evolution.

P0951. Asexual genetic variability in Agavaceae

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Agaves are succulent monocot plants rich in fibers, sugars and other important compounds. Molecular markers (AFLP and ISTR) were used to study genetic diversity in different Agavaceae plant samples. The comparison of the banding pattern between the mother plant and sucker derived daughter plants showed that genetic variability is also introduced during asexual reproduction in these species. Phylogenetic relationships among the Agave species were investigated using Bayesian inference with 3,000,000 generations, 4 independent Markov chains run, tree sampling every 100 generations and burnt after 300 trees. The Maximum Posterior Probability (MPP) tree shows the existence of four different taxonomic groups, with indication about the need of reclassification of some agaves. This asexual variability open the possibility for genetic improvement, as has been done in henequen.

P0952. Flowering inducers and repressors in rice

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Rice is a short day flowering plants. During the most of the vegetative growth phase, light acts as a repressor of flowering. We are investigating regulatory pathways that control the flowering signal pathways in rice. A MADS box gene, *OsMADS50*, is an inducer of flowering, acting very early stage of the plant development and the induction is long day dependent. Another MADS box gene *OsMADS56* is appeared to be a repressor of

flowering signal. They act to control expression of *OsMADS14*, which is a key transcription factor that induces flowering in rice. In addition to these MADS box genes, CO-like genes also play critical roles in controlling flowering time. *Hd1* is known to function for a repressor under long days and an activator under short day. We isolated a constitutive repressor *COL4*, which displays a circadian expression pattern opposite to that of *Hd1*. We also identified another CO-like gene, *COL8*, which acts as a flowering inducer. We are investigating interactions between these regulatory elements and how they control the downstream gene *Hd3a*.

P0953. *OsDAPD3*, a double AP2 domain gene, plays a major role in development of spikelet in rice

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In higher plants, the aboveground structure is determined by the two antagonistic functions of the shoot apical meristem (SAM), the maintenance in an indeterminate state and the successive organ formation. In this study, we report identification of the *OsDAPD3* gene as the main regulator in spikelet meristem transition to floral meristem. The gene encodes a putative transcription factor carrying two AP2 domains. The gene is expressed in the all the tissue examined. In *DAPD3* knockout plants, transition from spikelet meristems into floral meristems was delayed, resulting in production of more rudimentary glumes in alternative phyllotaxy. In addition, the empty glumes were transformed into the lemma/palea-like organs due to the increased expression of the *OsMADS1* gene. The lodicules were altered into the lemma/palea-like organs. These results indicate that *OsDAPD3* play a major roles in the transition of spikelet meristem to floral meristem and in floral organ specifications.

P0954. Petal Organ-Identity Genes Are Expressed in Petaloid Bracts of Dogwood

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The Dogwood tree *Cornus kousa* is known for its specialized bracts that surround the inflorescence and recall the bright, attractive petals of a large flower, even upon anatomical inspection. Genes from three classes (A, B and E) work in combination to specify a petal according to the ABC(DE) model of flower development, which is especially well-supported among the eudicots, of which *Cornus* is a member. Moreover, overexpression of an A and/or E class gene along with the two B class genes has been shown to be sufficient to convert vegetative leaves into petals in *Arabidopsis*. We have isolated members of these highly-conserved classes of petal-organ-identity genes from *C. kousa*: the A-class gene *CkAP1*, the B-class genes *CkPI* and *CkAP3*, and the E class gene *CkSEP*. We used RT-PCR to detect gene expression in bracts, leaves (negative control) and flowers (positive control). All four of these *C. kousa* genes are expressed in flowers, and none are expressed in leaves. In petaloid bracts, at least the A-class and two B-class genes are expressed, suggesting that these petal genes are turning leaves into 'petals' in this species.

P0955. An EST library from developing fruits of a Hawaiian endemic mint, *Stenogyne rugosa* (Lamiaceae, Lamiaceae): characterization and resources

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The morphologically diverse Hawaiian endemic mints include two genera with unusual fleshy fruits. An EST library was constructed from developing fleshy fruits of *Stenogyne rugosa* to develop genetic markers for lamioid mint research, as well as for comparison with Floral Genome Project and other unigene sets. 628 unigenes were derived from 904 high quality ESTs. Many unigenes were putative homologues of genes involved with reproductive development, e.g. *CAF*-like DEAD box, *EIN2*, *FCA*, *GI*, *LD*, *LUG*, *SYD*, *TFL1*, *UFO*, and *YABBY1*. Interestingly, *FCA*, *GI*, and *LD* are timing genes that promote flowering, whereas *TFL1* is a flowering inhibitor. Among the unigenes, >40 microsatellites (SSRs) were identified of which >50% were trinucleotide repeats. 8 SSRs could be annotated to reproductive development, 7 to RNA

and protein processing, and 3 to transcription factors. Given that allelic repeat length variation in developmental genes of other organisms has been linked with morphological evolution, these SSRs may be useful for analyses of phenotypic differences of lamioid mints.

P0956. MADS about perianth morphology in *Impatiens* and *Marcgravia*

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The balsaminoid Ericales consist of the families Balsaminaceae, with its core genus *Impatiens*, Marcgraviaceae and Tetrameristaceae. Judging from the long branch lengths, Balsaminaceae and Marcgraviaceae have probably evolved independently over a long period of time. This has hampered the robust reconstruction of the relationships in the balsaminoid clade and leaves us without clear morphological evidence of their evolutionary relations. Comparisons of the genes controlling flower development in *Impatiens* and *Marcgravia* can help to bridge the morphological gap between the two lineages. *Impatiens* has a petaloid sepal spur and *Marcgravia umbellata* has greenish sepals and petals. According to the quartet model of floral development, AP1, AP3, PI and SEP MADS-domain proteins control petal development in *Arabidopsis*. We have cloned several putative orthologues of the corresponding genes in *Impatiens hawkeri* and *Marcgravia umbellata*, and we report on their molecular evolution and functional diversity as indicated by expression patterns. We integrate these data with phylogenetic analysis of the balsaminoid clade, and observations of floral ontogeny.

P0957. AGAMOUS-like Genes Suggest Staminal Origin for the Perianth of Lauraceae

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The perianth of Lauraceae typically consists of two whorls of morphologically similar tepals. One frequent exception is a dimorphic perianth due to reduction in the size of the outer tepals. To determine whether orthologues of A, B, and C-class MADS-box genes have played a role in this morphological shift, their expression levels were compared across the dimorphic perianth of *Persea borbonia* and the more typical perianth of *Persea americana*. Our results indicate that A- and B-class orthologues are expressed at comparable levels in both perianth whorls of the two species, but AG (C-class) and SEP3 (E-class) orthologues are absent from the outer tepals of *Persea borbonia*. Based on our results, we hypothesize: 1. A role for AG- and SEP3-like genes in shaping perianth morphology is indicated by the coincident shift to a dimorphic perianth with loss of their expression in the outer tepals of *Persea borbonia*. 2. In *Persea*, sepaloid outer tepals and petaloid inner tepals are likely specified through action of A+B and A+B+C+E class genes, respectively. 3. The gene expression profile of the petaloid tepals of *Persea*, and perhaps other Lauraceae, suggests staminal derivation.

P0958. A comparison of floral morphology and physiology genes in an adaptive radiation

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We aim at testing the impact of flower morphology versus physiology genes in the process of speciation in the Macaronesian Sempervivoideae as a classic example of an island radiation. Substitution patterns of genes affecting the phenotype, e.g., flower morphology genes (AP1, AP3) and physiology genes (e.g. PEPC, TIP), are analysed. Increased mutation rates are expected, when comparing the adaptively radiated island clade to its non-radiated mainland sistergroup. This would indicate positive selection acting upon these gene classes. Analysing these genes that are potentially involved in the evolution of floral morphological or physiological key characters enables us to distinguish between different forces driving speciation. The comparison of evolutionary rates between these genes presumably affecting reproductive isolation and physiological adaptation will yield an indirect measurement of the degree to which these forces are linked to species diversification in this plant group.

P0959. The Molecular Basis for Convergent Loss of Floral Anthocyanins in *Aquilegia* (Ranunculaceae)

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Understanding whether phenotypic convergence extends to the molecular level is a basic question in evolutionary biology. Based on a highly resolved AFLP phylogeny for the North American *Aquilegia* clade, loss of floral anthocyanins (A-) has occurred independently at least seven times. These losses are usually associated with a transition to hawkmoth pollination and are inferred to be adaptive. We investigated whether variation in the expression of six structural and two regulatory genes in the anthocyanin biosynthetic pathway (ABP) could be responsible for the convergent (A-) phenotype in six species. We found evidence of expression in the first two enzymes in the ABP among the six A-species. Loss of expression in the remaining six genes however, varied among species and could account for the A- phenotypes. Most species showed different patterns of expression loss indicating that convergence usually does not extend to the molecular level during loss-of-function adaptations. These results emphasize the significance of variation in expression patterns for producing convergent adaptations during a rapid radiation.

P0960. Floral development in *Persea americana* (avocado): the male mystery in Lauraceae.

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Flowers of Lauraceae are small and consist of organs in distinct, **trimerous whorls**, with clearly **differentiated organ identities**. This syndrome is also found in other groups of magnoliids (e.g., Aristolochiaceae, Annonaceae) and in monocots. In Lauraceae, a **pair of nectar-secreting appendages** occurs at the base of the third whorl of stamens. Are they best considered accessory staminodes? Or are they additional pollen sacs, implying that the stamens are branched? The initiation sequence of the androecium and nectary-like or stamen-like structures outside of the androecium could help to answer these questions. The **delimitation of the androecium** towards the gynoecium and the perianth is discussed with respect to other basal angiosperms. The reliability of ***in situ* hybridization of an AGAMOUS homolog** (MADS-box gene required for the organ identities "stamen" and "carpel") is critically discussed. The expression of a single AGAMOUS homolog in the tepals may be insufficient to infer their homology with stamens, but may instead hint at the evolutionary processes of flower miniaturization and multiplication.

P0961. TEPAL-LIKE BRACT gene of *Fagopyrum esculentum* represents a new class of genes which states lower perianth boundary.

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A genetic and morphological study of new mutant of *Fagopyrum esculentum* (common buckwheat) has been performed. *Fagopyrum* wild type plants have a cymose partial inflorescence bearing hyaline scale-like bracts. The flower consists of 5 petaloid tepals, 8 stamens and 3 carpels. The morphological change in mutant line concerns the bract structure; no other significant difference is found. The bracts of mutant plants resemble wild-type tepals, so the line is called *tepal-like bract* (*tlb*). The likeness of wild type tepals and *tlb* bracts is confirmed by scanning electron microscopy. This feature is inherited as a monogenic nuclear recessive trait. Following the modified ABC-model petaloid tepals are mediated by the combination of A and B class gene activity. The morphology of *tlb* lets us suggest that *TLB* represents a class of genes which restricts ectopic expression of floral meristem identity genes involved in tepal development. The mechanism of *TLB* action may be similar to the same of *SUPERMAN* in *Arabidopsis thaliana* which states an apical boundary of B class gene expression. The work is supported by RFBR No 04-04-49643, RPLSI No 1731.2003.4.

P0962. Cytoplasmic homeosis in wheat

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Pistillody, homeotic transformation of stamens into pistil-like structures, has been reported in the cytoplasmic substitution (alloplasmic) lines of bread wheat (*Triticum aestivum*) with *Aegilops crassa* cytoplasm. The induction of pistillody is suppressed by the *Rfd1* gene located on the long arm of chromosome 7B in wheat cultivar 'Chinese Spring' (CS). Because of the absence of *Rfd1*, the alloplasmic line of CS ditelosomic 7BS ((cr)-CSdt7BS) lacking the long arm of chromosome 7B exhibits pistillody in all florets, whereas the euplasmic CS ditelosomic 7BS (CSdt7BS) with normal cytoplasm forms normal stamens. The class B MADS box genes, *WPI* and *WAP3*, were expressed in the normal stamen primordia of CSdt7BS, but not in the pistil-like stamen primordia of (cr)-CSdt7BS, indicating that pistillody is caused by the change of the MADS box gene expression. To clarify the mechanism of the cross-talk between MADS box gene and cytoplasmic genome, we have identified genes specifically expressed in the young spike of the pistillody line by using the cDNA subtraction technique. As a result, a protein kinase gene was cloned as a player of the cross-talk.

P0963. The differentiation of perianth morphologies in monocotyledonous plants.

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The morphological transition of the first whorl from petaloid tepals into sepals occurs frequently during the diversification of angiosperms. The class B genes, *AP3* and *PI* in *Arabidopsis*, are required for the petal development in whorl 2, and its homologs have been isolated and characterized from various plants. A recent study on tulip indicates that the morphology of petaloid tepals in whorls 1 and 2 is consistent with the expansion of class B gene expression. To clarify the evolutionary transition between sepals and petals in monocots, we isolated class B genes from *Agapanthus*, *Muscari* and *Tricyrtis*, whose perianths consist of petaloid tepals in two whorls, and *Tradescantia*, *Commelina* and *Habenaria*, whose perianths are differentiated in sepals and petals. RT-PCR studies using dissected floral organs revealed the expansion of class B gene expression in whorl 1 in *Agapanthus*, *Muscari* and *Tricyrtis*, and a lack or low level of *DEF(AP3)*-like gene expression in whorl 1 in commelinaceous and *Habenaria* species. This suggests that *DEF*-like gene expression pattern may correlate with morphological transition from petaloid tepals into sepals in the first whorl in monocot.

P0964. The modified ABC model explains the development of the petaloid perianth of *Agapanthus praecox* ssp. *orientalis* (Agapanthaceae) flowers

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The floral diversity of angiosperms has profoundly changed the terrestrial biosphere and it is of interest to identify the genes that are responsible for these heritable phenotypic differences. The class B genes, which belong to the MADS-box gene family, play important roles in regulating the development of petals and stamens in flowering plants. *Agapanthus praecox* ssp. *orientalis* (Agapanthaceae) has petaloid sepals in whorl 1. To understand the molecular mechanisms of floral development in *Agapanthus*, we isolated and characterized the homologs of the *Antirrhinum majus* genes *GLOBOSA* and *DEFICIENS* in this plant, designated by *ApGLO* and *ApDEF*, respectively. Expression analyses revealed that expression of *ApGLO* and *ApDEF* were observed in whorl 1 as well as in whorls 2 and 3. Moreover, the flowers of transgenic *Arabidopsis* plants that ectopically expressed *ApGLO* formed petal-like organs in whorl 1. We found that the class B genes of *Agapanthus* had the same expression pattern as those of tulip and lily. These observations indicate that the mechanism of flower development in *Agapanthus* follows the modified ABC model.

P0965. Diversity and evolution of Cycloidea-like genes in Papaveraceae.

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Floral symmetry in Angiosperm is supposed to have evolved as a response to specialized pollinators. Two main types of symmetry are recognized, actinomorphy or polysymmetry, and zygomorphy or monosymmetry. Zygomorphy would have evolved recurrently from actinomorphy, which rises the question of the homology of the molecular mechanisms involved.

Molecular developmental genetic studies in Snapdragon have shown that two paralogues, *Cycloidea* (*Cyc*) and *Dichotoma* were responsible for the zygomorphic wild-type flower. The molecular evolution of *Cyc*-like genes has been investigated in several Angiosperm derived taxa as related to floral symmetry. We present here a study of the evolution of *Cyc*-like genes in a primitive Eudicot taxon, the Papaveraceae that encompasses species with diverse floral symmetry. Two to three *Cyc*-like genes were found in 8 Papaveraceae species, corresponding to three homology groups. Homology of these genes with those of other taxa, and relationship with floral symmetry will be discussed.

P0966. Isolation of class B genes from *Muscari* and expression analysis using laser microdissection system

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In higher eudicot, the floral organs consist of four different whorls, containing sepals, petals, stamens and carpels. In contrast, the two whorls of perianths in many monocot flowers are almost identical petaloid organs, called tepals. To explain this flower morphology, the modified ABC model was proposed; two types of class B genes, *DEF*- and *GLO*-like genes, are expressed in whorl 1 as well as whorls 2 and 3, so that the organs of whorls 1 and 2 are the same petaloid structure. Although this model may apply to tulip and other Liliaceae as well, the class B genes were not expressed in whorl 1 in asparagus. In order to investigate whether the class B gene expression in asparagus is the common character in related species, we isolated and characterized the class B genes from *Muscari armeniacum* (Asparagales). We isolated two *DEF*- and three *GLO*-like genes from *Muscari* by RACE method. Total RNA was isolated from dissected floral organs by Laser microdissection system and RT-PCR was performed. The result showed that the class B genes were all expressed in whorl 1 as well as in whorls 2 and 3, which supports the modified ABC model in *Muscari*.

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P0967. Pod and seed morphology in Iranian species of *Sophora* and related taxa

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Pod and seed morphology are characters sets used to assess the relationships among Iranian species and subspecies of the tribe Sophoreae (Leguminosae: Papilionoideae).

In *Sophoreae* tribe fruits are extraordinarily diverse. In addition to obvious macro-morphological characters of pod and seeds such as size, shape, colour, wall thickness, positioning, type of pod opening and number of seeds per legume micro-morphological characters of pods and seeds can be useful for taxonomists.

In this study mature pods and seeds of each species and subspecies were collected from various geographic region of Iran. The morphology of the seeds and pods was studied using a zoom binocular light microscope and scanning electron microscopy. Photomicrographs were taken under different magnification. A number of macroscopic characters were also scored. The key summarized these data. Macro- and micro-morphological studies of pods and seeds of Iranian members of tribe *Sophoreae* showed some characters may support the identification of the species and varieties of *Sophora* and related genera.

Keywords: Leguminosae, pods, seeds, morphology

P0968. Verbenaceae

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Brazilian species of *Lantana* are included in four sections. The genus can be defined by the presence of 1 or 2 pyrene per fruit and succulent mesocarp (Schauer 1847). Specimens from 65 herbaria were examined (B; BM; BR; CEN; CEPEC, G; HAL; HUEFS; IBGE; INPA; IPA; K; LINN; M; MBM; MG; NY; OXF; P; R;

RB; SP; SPF; TEX; UB; UEC; US and W among others). Cladistic and morphological analyses suggest that *Lantana sensu* Schauer (1847) is polyphyletic and the circumscription of Chamisso (1832) would be preferable. This latter circumscription includes species with only one pyrene per fruit and no infrageneric divisions. Of the 85 names originally described, 21 species were recognised, 16 taxa were excluded, 32 new synonyms were proposed, and 6 new combinations were made. Fifteen names were lectotypified, one neotypified; 4 nomina nuda were detected, 2 later homonyms and 1 superfluous name were found. Eastern Brazil was the region with higher species number (18, of which 9 endemics), and is the main centre of species diversity in the country. The Atlantic Forest is especially rich in endemics (5 out of 9 species). Twelve out of the 21 species are exclusive to Brazil. (CNPq, UFEs)

P0969. The reproductive strategies of *Strobilanthes glandulifera* (Acanthaceae)

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Monocarpic and mass flowering are widely known in the tropical species of *Strobilanthes sensu lato*. *Strobilanthes glandulifera*, endemic to the Okinawa-jima Island is a single monocarpic species in Japan and a six years' mass flowering cycle was recognized. In the chance of mass flowering, which begun from November 2003, we examined reproductive strategies of the species, such as the ability of autogamy, duration of fruit maturation, seed germination rate and growth, viability of individuals after fruit set etc., using pot cultivated materials in the. Additional observation was carried out in the natural populations in the Okinawa-jima Island. As a result, individuals bagged with nylon net could produce fruits and the obtained seeds well germinated. The duration of fruits maturation was very variable. Most of the individuals were died after fruits set but the rest of them that left vegetative shoots survived and flowered in the following winter.

P0970. The genus *Lepiota* in Azerbaijan

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The genus *Lepiota* (Basidiomycota, Agaricales) comprises approximately 400 species that produce epigeous mushrooms with lamellate hymenophores. Species of the genus are widespread and have been recorded in deciduous forests, meadows and parks. Based on herbarium materials and recent collection 23 species: *L. acutesquamosa*, *L. adulterina*, *L. brunneoincarnata*, *L. castanea*, *L. clypeolaria*, *L. cortinarius*, *L. cristata*, *L. cygnea*, *L. eriophora*, *L. felina*, *L. fulvella*, *L. helveola*, *L. liliacea*, *L. micropholis*, *L. oreadiformis*, *L. pulverulenta*, *L. rosea*, *L. serena*, *L. setulosa*, *L. subalba*, *L. subgracilis*, *L. tomentella*, *L. wichanskyi* were identified on morphology. Most of species were collected from south part of Azerbaijan. *Lepiota fulvella*, *L. tomentella* were predominant in the north of country. Some of species are edible, but they are not in use by population because of small carpophores. The species of *L. acutesquamosa*, *L. brunneoincarnata*, *L. cristata*, *L. liliacea* are poisonous. *Lepiota brunneoincarnata* is widely spread and many incidents of poisoning with this fungus have been reported in the country.

P0971. Biological peculiarities of introduced species of the genus *Sedum* L. (Crassulaceae DC.)

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The abstract submission is devoted to the study of biological properties of the species of the genus *Sedum* L. The collection of the genus *Sedum* L., including 40 taxons, has been created in the O.V. Fomin Botanical garden of the Kyiv National University named by Taras Shevchenko. The rhythms of seasonal development of plants in culture has been investigated. The data of the anatomical structure of the vegetative organs of the species of the genus *Sedum* L. are established for the first. The peculiarities of seed and vegetative reproduction were revealed. The anti-virus activity of the species of the genus *Sedum* L. has been investigated. The results of investigations on content of biologically active substances - lectins in the vegetative organs of representatives of the genus *Sedum* L. are given.

It has been determined that the majority of the species *Sedum* L. which have been studied are perspective for cultivation in the

Forest-Steppe and Wooded Districts of Ukraine. The species *Sedum* L. have been selected for the use in ornamental gardening and phytoreclamation.

P0972. Umbelliferae of Russia

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Russia, world's largest country, occupies the area of 17075 thousand sq. km (~13 % of earth surface) in N & NE Eurasia. Diversity in Russian Umbelliferae (U) is not rich for such territory (109 genera, 298 spp., ~ 2.5% of country flora). In European Russia (50 genera, 87 native spp.; the biggest genera - *Seseli* - 9, *Chaerophyllum* - 6, *Bupleurum* - 5), and W Siberia (38 genera, 57 spp., excluding southern mountains; the biggest genera - *Bupleurum* - 6, *Seseli* - 5) widely distributed boreal Euro-Siberian U taxa prevail. More original U are distributed in N Caucasus (77 genera, 175 spp., the biggest genera - *Heracleum* - 17, *Bupleurum* - 15, *Chaerophyllum* - 11, *Seseli* - 9, *Pimpinella* - 7), Altay-Sayan mountains in Siberia (30 genera, 46 spp.), as well as in S part of Russian Far East, belonging to E Asian floristic region (34 genera, 65 spp.; the biggest genera - *Angelica* - 11, *Bupleurum* - 8, *Ostericum* - 5). The only endemic genus in Russian U is *Magadania*; genera *Arafoe*, *Mandenovia*, *Sajanella*, *Tamamschjanella*, *Symphyloloma* are subendemic; there are only 12 endemic species. Complete compatible descriptions, determination keys and dot maps were compiled for regional monographic treatment.

P0973. Molecular systematics of Campanulaceae subfam. Lobelioideae

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Relationships within Lobelioideae were inferred based on DNA sequence variation in the *rbcL* and *ndhF* genes, the *trnL-F* region including the *trnL* intron, and the *trnL-F* intergenic spacer. *Lobelia* appears as highly paraphyletic, reconfirming earlier studies. Giant lobelioids from Hawaii, Brazil, Africa and Sri Lanka form a strongly supported group. Some species of *Lobelia*, that were earlier referred to the genus *Pratia*, are clustered together with *Isotoma*. In order to make *Lobelia* monophyletic, the genus should only comprise some 30 species native to North America, Europe and the Antilles. Those species are sister to a clade comprising Central American and Antillean species of *Lobelia*, *Heterotoma* and *Hippobroma*. The analysis shows for the first time that the moss-like genus *Lysipomia*, native to the Andean Páramos, is sister to a group comprising the Neotropical shrubs *Burmeistera*, *Centropogon* and *Siphocampylus*. Moreover, *Centropogon* and *Siphocampylus* are not monophyletic. Evolutionary and biogeographical aspects are discussed.

P0974. New Caledonia: A hotspot for dioecy

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The unusually rich flora of New Caledonia includes over 3,200 indigenous species of seed plants, of which approximately 80% are endemic. It appears to be a distinctive combination of relict Gondwanan lineages and more recently evolved ones. Using the literature, herbarium specimens, and field observations, we determined sexual systems for 98% of the indigenous seed plant flora. We found remarkably high incidences of dioecy. Of the 44 species of gymnosperms, 70% are dioecious and the remaining 30% are monoecious. The distribution of sexual systems among angiosperms (3,095 species) is 19.5% dioecious, 0.7% gynodioecious, 0.4% androdioecious, 13.6% monoecious, 4.1% andromonoecious, and 61.6% hermaphroditic. For angiosperms, dioecy is over-represented among endemics, woody and rainforest species, and the Gondwanan component of the flora. Almost 90% of the dioecious angiosperms have at least three of four ecological traits appear to enhance the success of dioecious lineages. With over 4% of the world's dioecious species, the flora of New Caledonia provides a rich and important new source of information on the origins and maintenance of dioecy.

P0975. Biodiversity of the vascular plants on the Russian Far East

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The natural flora of the Russian Far East (RFE) covers 4347 species of the vascular plants from 979 genera and 176 families (Kozhevnikov, 2003). The indigenous species complex is presented by 3686 species from 789 genera and 169 families. The adventive (alien) species complex includes 661 species from 334 genera and 68 families.

Flora of RFE belongs to floras of Cyperaceae type. The Endemic element includes 470 species from 147 genera and 45 families. There are 7 endemic genera - Microbiota, *Acelidanthus*, *Miyakea*, *Ermania*, *Astrocodon*, *Popoviocodonia* and *Magadania*. The portion of main 10 families in indigenous species complex of RFE varies from 76-78% in arctic zone to 50% in nemoral zone. Adventive index changes from 6.1% in Magadanskaya District to 22.1% in Primorsky Territory.

According to structure of taxonomical spectrums two main groups of administrative areas on RFE were revealed. The first group situated in continental part of RFE and the second - in its maritime parts (Kamchatskaya and Sakhalinskaya Districts). The flora of Magadanskaya District has got intermediate position.

P0976. Two ivy (*Hedera* L., Araliaceae) species from the classic and geometric morphometrics points of view

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Two ivy species from Russia and Ukraine, *Hedera helix* and *H. colchica* (Araliaceae), are noticeably different in ploidy and micromorphological characters (hairs), but hardly distinguished in field conditions if only morphological characters used. We employed the simultaneous multivariate analyses of 8 metric leaf characters and three different approaches in geometric morphometry (landmark analysis via thin-plate splines and two variants of the elliptical Fourier analysis of the leaf outlines, totally 810 plants were measured) to test if there is the differences between these species in morphology and which of these methods is most suitable for taxonomic purposes. Our results show that only landmark-based method could clearly distinguish these species. Several of localities contain the forms with intermediate characters, this could be evidence of introgression between these two species. The samples of "*Hedera taurica*" from Crimea do not show any differences from typical *H. helix*.

P0977. A study on the varietal differences in the vegetative morphology of Citrus spp

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Seven varieties of Citrus spp were sampled for studies to assess varietal differences in their vegetative morphology, and to develop a taxonomic key that will simplify the classification of this angiosperm plants. The seven varieties used included Nigerian Green Orange[Citrus sinensis L. Osbeck], Sasuma[Citrus nobilis Var Unshiu], Shaddock[Citrus maxima], Grape[Citrus paradisi Mact], Tangerine[Citrus reticulata Blanco], Tangelo[tangelo*pomello] and Rough lemon[Citrus limoni]. A twig was cut from the tree of each variety under studies and 10 leaflets were abscised and randomly sampled for biometric analysis. Length, width and petiole length of the leaflets were also measured and the data obtained were statistically analyzed. A taxonomic key was formulated for identification of the species studied using their morphological characters and the measured parameters. It is possible that a more detailed key could evolved with the study of all Citrus species and could be used in taxonomic studies of other angiosperm.

P0978. Genetic differentiation and relationship of populations in the *Aconitum delavayi* complex (Ranunculaceae)

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RAPD markers were employed to examine the differentiation of 18 populations representing all species of the *Aconitum delavayi* complex distributed mainly in Hengduan Mountains of China. The PCO and UPGMA analyses of RAPD data indicate that the

complex comprises three different clusters. The first cluster consists exclusively of *A. episcopale* populations, which indicates that *A. episcopale* is a very distinct species, in good agreement with our previous allozyme and ITS data. The second includes one *A. campylorrhynchum* population and all the *A. henryi* populations from the northern Hengduan Mountains and its neighbor areas. The third comprises the populations of *A. delavayi*, *A. stapfianum*, *A. tuguncunense* and *A. campylorrhynchum* from the southern Hengduan Mountains. The little genetic differentiation among populations of *A. stapfianum*, *A. delavayi* and *A. tuguncunense* suggests that they would better be treated as a single species. The fact that *A. campylorrhynchum* populations appear in two different clusters demonstrates that *A. campylorrhynchum* should be treated as two different species.

P0979. Phylogeny of the genus *Anthurium* Schott (Araceae): Preliminary results

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Anthurium, with approx. 700 species, all from the New World tropics, is the largest genus in Araceae, and is currently divided into 18 sections mainly distinguished by leaf morphology and habit. Species with lobed leaves have been placed in sections *Schizoplacium*, *Semaeophyllum* and *Dactylophyllum*, however, a molecular phylogeny using chloroplast markers shows that lobed leaves have evolved independently more than three times. Preliminary results also indicate that other sections may not be monophyletic whereas some tend to hold together (e.g. bird-nest *Anthurium*, section *Pachyneurium*). These data thus suggest that the current morphological sectional classification of *Anthurium* needs re-evaluation. Further molecular and morphological work is underway in order to develop a robust phylogeny for the genus on which a revised classification can be based. The phylogeny will also help clarify the evolution and diversification of this ecologically important genus.

P0980. Phylogeny of *Anagallis*

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Anagallis (Myrsinaceae), with 31 recognised species, includes small herbaceous, erect or decumbent plants diagnosed by the presence of circumscissile capsules. Most species have a limited distribution, several in tropical East Africa, others in Madagascar, South Africa or the Mediterranean region. A few species are more widespread, e. g. *Anagallis arvensis* or "Poor man's weather glass". Previous molecular studies place *Anagallis* nested within *Lysimachia* but in two different clades, raising questions regarding the monophyly of the genus. Our present study of nuclear and cpDNA data supports *Anagallis* as a monophyletic group, but only if *Asterolinon* and *Pelletiera* as well as *Lysimachia nemorum* and *L. serpyllifolia* are also included. In *Anagallis* two monophyletic groups appear with strong support, the first supported by several unique morphological floral characters while synapomorphies for the second group are less obvious, despite an overall similarity between its species.

P0981. Multivariate morphometric analysis of the *Caltha palustris* L. complex from the representative parts of its European range

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Taxonomical-chorological studies on the *Caltha palustris* L. complex were carried out based on the material of North and Central European provenance. Detailed analysis of the intra- and interpopulational variability, based on several morphological features of the specimens from various parts of the species' range, allowed to determine the limits of this variability and to assess a taxonomical status of the discerned morphotypes. Numerous morphologically intermediate forms were also revealed. Several techniques of the numerical analysis have been applied (PCA, CA, DA, ANOVA) to characterize the variability of the material studied. Based on the data obtained for the variability as well as on the geographical distribution of the whole complex, its taxonomical treatment was proposed and, for the taxa distinguished, the

pattern of the horizontal and the vertical ranges was identified. As a result, the following taxonomy of the complex has been adopted:

C. palustris L. ssp. *palustris*

ssp. *cornuta* (Schott, Nyman & Kotschy) Hegi

ssp. *radicans* (T. F. Forst.) Syme

C. laeta Schott, Nyman & Kotschy

P0982. Reappraisal of Sectional Classification of *Anthurium* (Araceae)

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A total of 1024 species are now known in the genus *Anthurium* (Araceae). This represents a 235 % increase in numbers of species from the 305 treated in last revision of the genus by Engler in 1905. The current sectional classification of *Anthurium* distinguishes 18 sections, however this reappraisal has placed many more species in section *Porphyrochitonium*, eliminated section *Oxycarpium*, greatly modified the understanding of section *Urospadix*, added the new section *Decurrentia*, accepted *Tetraspermium*, *Dactylophyllum*, *Gymnopodium*, *Pachyneurium*, *Polyphyllum*, *Leptanthurium*, *Cardiolonchium*, *Chamaerepium*, *Calomystrium* and *Belolonchium* as seemingly natural sections, and suggests *Porphyrochitonium*, *Xialophyllum*, *Polyneurium*, *Urospadix*, *Episeiostenium*, *Digitinervium*, *Semaeophyllum* and *Schizoplacium* as sections in need of more investigation.

P0983. Taxonomic diversity and conservation of genus *Trichosanthes* in India

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The genus *Trichosanthes* (Cucurbitaceae) is represented by 21 species in India. *Trichosanthes amalaiensis* Bedd., *T. perrottetiana* Cogn., *T. villosula* Cogn., *T. cuspidata* Lam. are endemic to South India. *T. himalensis* var. *glabrior* Clarke, *T. ovata* Cogn. and *T. tometosa* Chakravarty are endemic to Khasi hills (Meghalaya), Sikkim and Nagaland respectively. *Trichosanthes* species are found in wild conditions except *T. cucumerina* var. *anguina* L. and *T. dioica* Roxb. which are cultivated as vegetable crops. Plants are extensive climbers. Most of the species are dioecious except *T. cucumerina* var. *anguina* and *T. horsfieldii* Miq. which are monoecious. Flowers are generally white and are characterised by the presence of frills. The pollen grains are spherical and triporate. Fruits are fleshy, globose, ovoid or fusiform, indehiscent and many seeded. *T. dioica* possess round and smooth seeds. *Trichosanthes* exhibits diversity at specific and infraspecific levels. This diversity needs to be conserved especially in *T. dioica* where varieties are known only under cultivation.

P0984. Phylogenetic relationships in Aponogeton (Aponogetonaceae) based on chloroplast DNA sequence data, with implications for the evolution of fenestrated leaves in lace plants

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A phylogenetic analysis of the chloroplast DNA of Aponogeton species was undertaken. The Aponogetonaceae includes only Aponogeton, which is an aquatic genus with about 45 species distributed throughout the tropical and subtropical regions of Africa, Asia, and Australia. The lace plant, Aponogeton madagascariensis, is characterized by fenestrated leaves. Our anatomical observations suggest that its three varieties are divided into two types according to the process involved in perforation formation. This study examined the phylogenetic relationships in Aponogeton to reveal the evolution of fenestrated leaves. In the molecular phylogenetic tree constructed using the chloroplast genes *rbcL* and *matK*, the three varieties of lace plant do not form a monophyletic group. Therefore, fenestrated leaves arose more than once in Aponogeton. In addition, leaf morphology and geographic distribution are discussed based on character-state reconstruction.

P0985. Taxonomic studies in Indian *Curcuma* L.: what causes such confusion in the genus?

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The genus *Curcuma* L. (Zingiberaceae) with over 100 species is distributed in tropical Asia with the highest diversity in India and Thailand. About 30 species have been previously recognized in India, but the number of published names has been neglected and over 60 names have been published for Indian taxa. Many of these names may be synonyms, but some of the earlier proposed synonyms are incorrect and need critical reconsideration. Several species were described 200 years ago and their true identity is not always clear. Substandard descriptions, lack of type specimens, the need to study fresh flowering material, the overall resemblance of many species and high intra-population variability of seed-setting species with a wide distribution makes the study difficult. This poster presents preliminary results of our extensive fieldwork conducted between 2000-2004 as part of a revision of this economically and ornamentally important genus for India.

P0986. The carpology and systematic relationships of the subfamily Hydrocotyloideae (Apiaceae)

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As a result of our comparative morphological and anatomical study of fruits in numerous representatives of the subfamily *Hydrocotyloideae* (*Apiaceae*), tentative corrections to Drude's system were proposed. These changes mainly relate to the size of tribes and subtribes: thus, we consider advisable to elevate South African endemic *Hermas*, possessing a fruit with a special secretory system type, to tribal rank. We also propose to exclude Australian genus *Actinotus* from the *Apiaceae*, because the way of ovary development in it and some structural traits of its highly specialised one-seeded indehiscent fruit are absolutely uncharacteristic for this family. On the basis of significant carpological differences between the subfamily *Hydrocotyloideae* and subfamilies *Saniculoideae* and *Apioidae*, as well as the analysis of available data on palynomorphological, embryological, biochemical features, the concept of *Hydrocotyloideae* as a separate family *Hydrocotylaceae* Hylander seems thoroughly acceptable.

P0987. Phylogenetics relationships of Ebenaceae : inferred from four regions of plastid and *ncp* glutamine synthetase

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Phylogenetics relationships in Ebenaceae were investigated by parsimony analysis of combination of DNA sequences of plastid *matK*, *trnK* intron, *trnL*F region, *trnSG* spacer and *ncpGS*. Preliminary results clearly show that Ebenaceae are monophyletic. *Lissocarpa* is sister to *Euclea* and *Diospyros*. Member of *Diospyros* section *Royena* are grouped together with *Euclea* as a separate clade. In *Diospyros*, subgeneric status of subgen. *Hierniodendron* with *D. maingayi* is confirmed, and is also supported by morphological evidence. However except this subgenus and the section *Royena*, all other species of *Diospyros* form a unresolved clade containing subgenera *Maba*, *Eudiospyros* and other species from Asia minor, North America and Africa. One African *Diospyros* species (*D. sp.* Eb205) is sister to this unresolved clade without a good support. Within subgen. *Eudiospyros* our results give better resolution at sectional level for sections *Capanulata*, *Ebenaster*, *Glutinosa*, *Kurzella*, *Lotus*, and *Ptychocylix*.

P0988. Reappraisal of *Diplolabellum coreanum* (Orchidaceae)

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The taxonomic treatment of a monotypic genus, *Diplolabellum*, which was described by Maekawa in 1935 based on *Oreorchis*

coreana Finet, has been in dispute by various authors. Since the distribution of this plant is very limited in Jeju Island of the South Korean Peninsula, the species has not been well studied. To reappraise *Diplolabellum coreanum*, ITS, *trnT-trnL* and *trnL-trnF* sequences were obtained from *D. coreanum*, several species of *Oreorchis* and related genera. In the sequence analysis, *Diplolabellum coreanum* was revealed to be closely related to one group of *Oreorchis*, which consists of *O. patens* and *O. fargesii*. Molecular data therefore suggest that the species should be treated as *Oreorchis coreana* rather than *Diplolabellum coreanum*, even though this species is distinct from the other taxa of *Oreorchis* in morphological characters such as callus, pedicel, column and caudicle.

P0989. Phylogenetic trends in fruit structure of Apiaceae

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With recent advances in molecular systematics of the Apiaceae at suprageneric level, it has become possible for the first time to interpret the remarkable diversity of fruit structure in a new way. The evolution of dorsally compressed fruits, vittae, loss of rib oil ducts and crystals, and reduced lignification of the endocarp appear to be unique events which help to define large groupings of genera within the family. There is great variability with regard to the shape of the two mericarps comprising the fruit, the configuration of wings (if present), the presence or absence of outgrowths on the mericarps, the shape of epidermal cells, the number of vascular bundles, the configuration of the rib oil ducts and vittae (if present), the presence or absence of crystals and their positions, the degree or lack of lignification of the endocarp, and the carpophore (and ventral bundle) configuration. This variability limits the diagnostic utility of many of the features (homoplasy seems rampant) but several general trends can clearly be distinguished.

P0990. The systematic significance of seed morphology in *Stenocereus* (Cactaceae)

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Seed morphology of 24 species of *Stenocereus sensu* Barthlott and Hunt was examined by scanning electron microscope. Quantitative and qualitative features were evaluated using phenetic analysis in order to identify groups of species. Two groups of species were distinguished based on morphological variations of seed size, luster, multicellular sculpture, cell-size, sculpture of periclinal walls, microrelief, position relative to rim and shape of hilum and micropyle complex. All studied species are keeled with isodiametric cells. *Stenocereus alamosensis*, *S. kerberi* and *S. beneckei* are unique among species of *Stenocereus* in having seeds with flat relief and lacking micro-relief. *Stenocereus aragonii* and *S. eichlamii* also have seeds with large size, glossy appearance, and lack of micro-relief as most *Pachycereus* species. *Stenocereus dumortieri* shares with the other *Stenocereus* species most seed characters, specially the coarse striated cuticle in all periclinal walls with *S. martinezii* and *S. quevedonis*. Congruence and discordance among characters for species groups in *Stenocereus* are discussed.

P0991. Phylogenetic analysis in Rhynchosporae (Cyperaceae)

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Rhynchospora is the most diverse sedge genus in the Neotropics. Kükenthal's revision recognized two subgenera (each of which was divided into 2-3 supra-sectional taxa called "pars"), 28 sections, and placed *Pleurostachys* outside of *Rhynchospora*. A phylogenetic analysis of the plastid gene *trnL-F* was conducted in order to evaluate phylogenetic validity of the separation of *Pleurostachys* from *Rhynchospora*, the monophyly of the subgenera, and the soundness of the "pars" level groupings. *Cladium mariscus* was used as the outgroup in this analysis of three species of *Pleurostachys* and 22 of *Rhynchospora*, representing 11 of Kükenthal's sections. Preliminary results indicate that *Pleurostachys* is distinct from *Rhynchospora* and that,

possibly, some species of *Rhynchospora* (i.e., *R. splendens*) should be placed in *Pleurostachys*. Within *Rhynchospora*, a large clade comprising most sections of subgenus *Haplostylis* is distinct. Subgenus *Rhynchospora* and the remaining sections of subgenus *Haplostylis* are poorly resolved but show some evidence of clustering into clades resembling some of Kükenthal's supra-sectional groupings.

P0992. Japanese *Oenanthe javanica* contains two biological species: evidence from chloroplast DNA

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Oenanthe javanica (Blume) DC. is a perennial herb well known as "Seven Spring Herbs" in Japan since Heian period of 8th century. Some morphological characters (leaf shape, length of umbel rays and so on) of *O. javanica* shows large intraspecific variation. However, this plant species has been treated as one species due to continuous morphological variation, especially in leaf shape. In Japan, some taxonomists recognized two subspecies, subsp. *javanica* and subsp. *linearis* in *O. javanica* based on their difference in leaf shape. We analyzed noncoding regions of cpDNA, rps16 intron and trnG (UCC) intron, of these intraspecific taxa growing in Japan and also observed their flowering period, habitat, and leaf shape. Two haplotypes of cpDNA were found in this species two ecological traits (flowering period, habitat) and morphological trait (cauline leaf shape) were observed in each haplotypes. These results showed that Japanese *O. javanica* can be divided into two independent species. *O. javanica* is widely distributed in Southeastern Asia, Taiwan, and China. We should investigate plant samples in these areas as well.

P0993. Biological varieties of plants in South Caucasus and their protection

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The problem of flora protection in South Caucasus has become more pressing. Periodicity of processes has become sharp and the results of it have become irresistible. As human affects to natural flora more increasingly, the problem of the protection of genofund is becoming more vital.

It was defined that 460 species out of more than 4500 in the flora need to be protected. Every tenth variety in the flora is endangered.

We must note with regret that the habitat of 28 species out of 140 that have been included in the "Red Book" is in Garabagh.

The only place that endemic species like *Scoroteria pulchra*, *Anemone kuznetzovii*, *Qladiolus halophilus* exist in nature is Garabagh. On the other hand *Stellaropsis maqakyanii*, *Salsola tamamschyane lactuca takhadzianii* are spread only in 2 places in nature-Nakhchivan and Armenia. The biological diversity of Caucasus can be protected by the joint efforts of the Caucasian scientists.

P0994. Studies on interspecies relationship of *Smilax china* complex based on Karyotypes and molecular data analysis

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Smilax china and their relatives distributed in Chi. Kor. and Jap., including 12 populations of *S. china* (one 2X, three 4X and eight 6X) and 8 relatives of *S. polycolea*(4X), *S. ferox*(6X), *S. lebrunii*(6X), *S. davidiana*(2X), *S. trinervula*(2X), *S. chingii*(2X), *S. glauco-china*(2X), *S. scobinicaulis*(2X) and *S. biflora*(4x) are studied using ITS, matK and isozyme. Results show *S. davidiana*, *S. trinervula* and *S. china* in Hubei have close relationships to polyploid species of the group and maybe diploid parents. *S. davidiana* & *S. trinervula*, *S. biflora* & *S. polycolea*, and *S. ferox* & *S. lebrunii* should be clear into the complex. *S. lebrunii* are more closer to 4X Jinfu *S. china* and *S. polycolea* distributed in same area. *S. china* var. *kuru* and *S. biflora* distributed in Okinawa islands are close to *S. trinervula* of China, however, 4x population in Jap. is sister to 4x and 6x populations in China. It is suggested *S. glauco-china* and *S. scobinicaulis* of black fruits are more different from *S. china* complex of red fruits. It is considered that polyploidy of the complex is of multiple origins and is allopolyploidy. A possible evolution and speciation are given in the paper.

P0995. Improving growth and productivity desert plants: Laticiferous plants.

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The semi-arid and arid regions of the world have vegetation rich and latex which helps it to overcome drought conditions on one hand and prevent browsing from the stray cattle. Arid region of India lies between 24 degree and 29 degree latitude and 70 degree and 76 degree longitude and covers 3,17,090 km square spread over seven states of Indian Union. Ninety percent if arid region about 2,85,580 km square is confined to north west India, covering most of the western Rajasthan. The present investigations were undertaken to characterise the plant species appearing at different stages of vegetation development. The vegetation of non-saline wastelands was determined and potential renewable sources of bio-energy were identified. Several plants had uses in traditional medicines. The detailed investigation of selected plants were carried out and different factors like nutrients and growth regulators affecting growth and productivity of desert plants were determined with special reference to laticiferous plants. Details shall be presented.

P0996. Immunological investigations of relationships of taxa within Apioideae (Apiaceae): correspondence and disagreement with DNA sequence data.

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The results of the immunological comparison of storage seed proteins and of DNA sequence analysis of *Apioideae* (*Apiaceae*) taxa mostly correlate with each other and revealed the pattern of evolution within the subfamily that poorly supports earlier suggested classifications based on morphological characters. Among main lineages within *Apioideae* supported by results received by both methods are: *Phyospermum*-group; *Parasilau-Komarovia-Hansenia-Notopterygium* group; *Caucalideae-Scandiceae-Dauceae-Thapsieae* group; *Smyrnium* group; Apioid-superclade; among smaller groups are *Apium-Anethum-Foeniculum-Petroselinum* group; *Heracleum-Pastinaca-Zosima* group and some other. But the inclusion of some taxa in the Apioid-superclade by DNA analysis was not confirmed by immunological data and *vice versa*, as well as the subdivision of the superclade. Discordant results were received concerning relationships and/or phylogenetic position of *Carum*, *Smyrnium*, *Ferula*, *Conioselinum*, *Ligusticum scoticum* and some other taxa.

P0997. Taxonomic treatment of *Salvia* Section *Incarinatae* (Lamiaceae)

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Salvia is a not well understood genus despite its economic and ecological importance. For a thorough understanding of the systematics and phylogeny of *Salvia* it is necessary to have taxonomic treatments of as many groups as possible, particularly those with taxonomic or nomenclatural problems. Of the 94 sections that Epling recognized in 1939 for subgenus *Calosphace*, 34 are mainly Mexican in distribution, but only section *Sigmoideae* has been recently studied. Section *Incarinatae* is treated, including its two species, *S. elegans* and *S. cinnabarina*, and their varieties status is discussed. *Incarinatae* has petiolate leaves, 3 to 6 flowers per semiwhorl, upper calyx lip five veined, corolla tube sulcate longitudinally near the base in *S. cinnabarina*, and exerted stamens from the corolla's upper lip. *S. elegans* distributed from the north of Mexico into Oaxaca and *S. cinnabarina* from Oaxaca into Central America. Several nomenclatural problems have been found particularly in *S. elegans*.

P0998. Phylotaxonomy of genus *Scabiosa* (Dipsacaceae) in East Asia.

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Genus *Scabiosa* is distributed mainly in temperate zone of Europe, Africa and Asia, and comprises ca. 90 spp. In Japan, *S. japonica* s.l. and *S. jezoensis* occur widely in seaside to alpine areas throughout the Japanese archipelago. The taxonomical review in Japanese *Scabiosa* revealed that *S. japonica* s.l. and *S. jezoensis*

have to be classified into different sections, though they have been treated as varieties of the same species until quite recently. *S. japonica* s.l. is classified into section *Scabiosa* by its cylindrical, deep and narrowly 8-sulcated epicalyx. This is the unique taxon among the East Asian species in this respect, and the species of the same section are distributed in Europe, Africa and central Asia, but never in East Asia. *S. jezoensis*, on the contrary, shows the same characteristics as in the section *Prismakena* that is distributed only in East Asia.

We reexamined the former taxonomic systems in *Scabiosa* based on morphology and habitat, and molecular analyzing using cpDNA and nDNA gave the new insights for speciation and dispersion of E-Asian *Scabiosa*. Furthermore we will discuss the phylotaxonomy of *Scabiosa* in East Asia and of Dipsacaceae in the world.

P0999. Genetic diversity of an endangered slipper orchid, *Cypripedium macranthos* var. *rebunense* and the impact of a sympatric relative, *C. calceolus*

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An endangered species, *Cypripedium macranthos* var. *rebunense* is distributed in the most northern small island in Japan. This species used to be collected illegally and is fragmented to several populations. We estimated genetic diversity of these populations using 13 allozyme loci. Genetic diversity in a small population is not different from those in large. The gene differentiation index (Gst) is 8.4%. These results show fragmentation of the populations would have occurred recently enough that genetic diversity has not decreased even in small population and genetic differentiation has not occurred among the populations. Eight plants of *C. calceolus* grow sympatrically in the protected area of *C. macranthos* var. *rebunense*. These plants doubt to be planted. We identified their hybrids using 8 allozyme loci, ITS2 and 4 cpDNA sequences. Six putative hybrids had both specific markers derived from two species. Judging from the result of cp DNA markers, *C. macranthos* was their mothers. Two plants have homozygotes of *C. macranthos* specific alleles in MNR and EST-3 respectively. It is indicating the backcross hybridization may have occurred with *C. macranthos*.

P1000. Carpoanatomy supports polyphyly in *Podistera* (Umbelliferae/Apiaceae) and generic status of *Orumbella*

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Sun, Downie & Hartman showed recently, on the basis of molecular analysis, that many N American Umbelliferae genera are not monophyletic. This refers to *Podistera*, the genus of dwarf alpine and arctic perennial polycarpic herbs. The systematics in American Umbelliferae was traditionally based on different morphological characters, and was developed without detailed analysis of inner fruit structure. American monographers even rejected the taxa which they referred to "carpological genera". Our carpological analysis of 3 *Podistera* species (*P. nevadensis*, the type of genus' name, *P. eastwoodae*, and *P. macounii*) revealed essential differences among the species in mericarp transsection form, rib form and structure, in the absence/presence of parenchyma cells with lignified pitted walls and in absence/presence of rib secretory ducts, as well as in style structure. For instance, Beringian (Alaska & Chukotka) endemic *P. macounii* differs considerably from *P. nevadensis*. The genus *Orumbella* Coult. & Rose is to be restored for this species. Russian and American *O. macounii* have similar fruit structure. In this case, carpological and molecular data are congruent.

P1001. Evolutionary patterns in the transition between the terrestrial and epiphytic habit in Orchidaceae

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Multi-gene phylogenies of Orchidaceae show that the terrestrial habit is plesiomorphic in the family. Four of the five orchid subfamilies are almost exclusively terrestrial, as are the tribes of "lower" Epidendroideae. Only within the "higher" Epidendroideae (which constitutes ca. 80% of all orchid species) do we find entirely obligate epiphytic lineages, and this habit defines 88% of those

genera and 92% of their species. But, there are a few higher epidendroid lineages that have secondarily reverted to a terrestrial lifestyle. To explore one example of this phenomenon, ITS and *matK* sequences were collected for 71 taxa of Malaxideae (a tribe containing both epiphytes and terrestrials) in order to develop a phylogenetic hypothesis for the genera and species. Highly resolved and supported cladograms indicate that the tribe is split into one clade of terrestrials and another of epiphytes, documenting that the reversal to terrestrial habit has occurred only once in Malaxideae. Whether or not a similar unilateral pattern is characteristic of other orchid clades remains to be investigated within the framework of robust phylogenetic hypotheses.

P1002. Phylogenetic and Biogeographic Relationships in *Polyscias* s. lat. (Araliaceae)

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"*Polyscias* s. lat." unites the paraphyletic genus *Polyscias* (~150 spp.) to 6 other genera of Araliaceae. Analyses using molecular data suggest an Australian-Malesian origin of the clade, but most species have insular Pacific or Indian Ocean distributions, and diversity is highest in New Caledonia and Madagascar. Thus, *Polyscias* s. lat. affords an opportunity to compare dispersal patterns between these two ancient, floristically-rich, continental islands. Results suggest multiple, independent dispersals to land masses in both oceans. In the Indian Ocean, independent dispersals led to separate Seychelles and Malagasy lineages, with secondary dispersals from Madagascar to the Mascarenes, Comoros, and continental Africa. In the Pacific, three independent dispersals from Australasia to New Caledonia can be inferred, with subsequent dispersals to other Pacific islands (e.g., Fiji, Vanuatu, Lord Howe Is.) in one of these radiations. At least two additional dispersals into the Pacific have been identified that do not include New Caledonian representatives.

P1003. Floral structure of *Ditassa burchellii* var. *burchellii* (Apocynaceae, Asclepiadoideae) with emphasis on glands

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The purpose of the present work is to show the floral structure of *Ditassa burchellii* Hook. & Arn. var. *burchellii* with special reference to the observed glands. It is a liane growing in mesophytic forest edges that bears tiny white flowers producing a characteristic scent. Inflorescences were collected, and mature flowers investigated using scanning electron microscopy and histological serial sections. Different types of glands were observed: **1. bracteolar and calycine colleters** are emergences exuding a sticky substance that lubricate and protect the developing floral buds; **2. papillose epidermis** are covered by non-glandular trichomes in the upper surface of the petals; **3. secretory palisade-like epidermis** of the gynostegium produces the translators; **4. secretory epidermis** also occurs on the filaments at special regions of the gynostegium and corona; **5. laticifers** are widely distributed on the floral organs - from sepals to gynostegium - forming a continuous system dispersed among the ground tissue. The glands variety reinforces the floral complexity presented by species of Asclepiadoideae. (CNPq)

P1004. Pyrrolizidine alkaloids in Apocynaceae

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Pyrrolizidine alkaloids and/or their N-oxides (PAs) are secondary compounds typical for several Asteraceae (e.g., *Senecio*, *Gynura*), Boraginaceae (e.g., *Heliotropium*, *Echium*), and Fabaceae (*Crotalaria*). However, several types of PAs have also been reported from a few species of Apocynaceae s.l.

Relationships of "PA-insects" (e.g., certain butterflies and moths) with Apocynaceae indicate that further taxa of Apocynaceae also contain PAs. These are reviewed and results of new chemical analyses are provided. Although they are insufficient to provide a conclusive picture on the distribution PAs in Apocynaceae, they suggest that studying further species seems rewarding; more comprehensive knowledge on the presence of PAs in species of Apocynaceae not only helps elucidating the evolution of insect-PA

relationships but likely will contribute to the systematics of Apocynaceae.

P1005. Gynostegium morphology of Mesechiteae (Apocynaceae, Apocynoideae) based on species of *Forsteronia*, *Macrosiphonia*, *Mandevilla* and *Mesechites*.

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In order to investigate morphological patterns in Mesechiteae and test the new circumscription of the tribe, the gynostegium structure of eight species belonging to *Forsteronia*, *Macrosiphonia*, *Mandevilla* and *Mesechites* as well as two species from *Secondatia* (as outgroup) were comparatively studied. The gynostegium of *Forsteronia*, *Macrosiphonia*, *Mandevilla* and *Mesechites* all have the same basic pattern: a proliferation of parenchyma cells in the style-head forms five projecting ribs that are united either only at their base or along their entire length to the stamens via cellular fusion. In *Secondatia*, on the other hand, the style-head has no projecting ribs and union is via unicellular trichomes of the connective, which are inter-digitated with the secretory epidermis of the style-head, without cellular fusion between the parts. The observed differences between the gynostegium morphology of *Secondatia* and that of the other genera agree with the new circumscription of Mesechiteae, supporting the inclusion of *Forsteronia* in the tribe and the transfer of *Secondatia* to Apocynaceae. (FAPESP, CNPq)

P1006. Molecular Phylogeny and Biogeography of the Secamonoideae (Apocynaceae s.l.)

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The poorly known Secamonoideae are nested in the Apocynaceae s.l. in which they are sister group to the subfamily Asclepiadoideae. This palaeotropic subfamily is represented by 7 genera with about 200 species showing an extensive morphologic diversification and an interesting Gondwanan distribution pattern. Madagascar is the main centre of radiation of this subfamily with about half of the known species occurring; the other taxa are distributed in Africa, Asia and Australia. Relationships within Secamonoideae are investigated by maximum parsimony and Bayesian inferences of molecular sequence data from the plastid trnL intron, trnL-F spacer, and the matK gene with its first trnK spacer. From this largest phylogeny of the Secamonoideae ever presented, we particularly put emphasis on phylogenetic relationships of the main genus *Secamone*. The next largest genus, *Toxocarpus*, appears to be closely related to *Secamone*. The biogeography of the Secamonoideae is also discussed for the first time showing no clear vicariance patterns between continents and islands, but instead repeated supposed long distance dispersals.

P1007. Phylogenetic analysis of the Cascabela-Thevetia complex (Plumerieae; Apocynaceae) based on morphology.

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Cascabela, Cerbera and Thevetia have a convoluted taxonomic history and controversial generic circumscription. Two solutions to this dispute have been proposed: 1) recognition of two genera, Thevetia (including Cascabela) and Cerbera, or 2) recognition of three genera, Cascabela, Cerbera and Thevetia. A morphological cladistic analysis was conducted aiming to solve this controversy. This analysis incorporated 12 genera and 22 species, including all species of Cascabela (4) and Thevetia (4), three species of Cerbera and at least one species of each of the remaining genera in tribe Plumerieae (sensu Endress & Bruyns). The matrix has 53 morphological characters, several of which are used here for the first time. The consensus of the most parsimonious

trees supports the monophyly of Cascabela, Cerbera and Thevetia only if *T. pinifolia* is transferred to Cascabela. Since Cascabela and Thevetia share a

most recent common ancestor, the results of this analysis are inconclusive regarding the recognition of one or two genera, but given the characters that support these clades we prefer to recognize two genera.

P1008. An evaluation of *Matelea* subgenus *Poicilla* (Apocynaceae - Asclepiadoideae - Gonolobinae): Parsimony analysis based on morphology

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The circumscription of *Matelea* (Apocynaceae - Asclepiadoideae) and submerged genera, such as *Poicilla*, *Poicillopsis*, and *Ptycanthera*, has been particularly complicated. Although many subgenera are likely ill-conceived and some submerged genera were recently re-instated, tests of the subgeneric concepts in *Matelea* have not been published. This study sought to test the monophyly of *Matelea* subgenus *Poicilla* sensu Woodson based on parsimony analysis of morphological characters and to evaluate the systematic implications. A data matrix was produced by appropriately scoring character states for thirty-two species based on study of herbarium specimens and the literature. The data matrices were analyzed using parsimony methods in PAUP 4.0*. Results of analyses showed *Matelea* subgenus *Poicilla* sensu Woodson to be paraphyletic. The narrower generic concepts of other authors, such as Grisebach and Schlechter, also appeared non-monophyletic, although additional resolution is needed.

P1009. Molecular Systematics of *Oxypetalum* R.Br. (Asclepiadoideae, Apocynaceae).

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The subfamily Asclepiadoideae is one of the largest in the Apocynaceae. The group is mainly tropical and subtropical, with a centre of diversity in South America. *Oxypetalum*, a group centred in Brazil, is the largest Neotropical genus of the subfamily (ca. 130 species). The genus is generally recognised by a combination of characters that include a rostrated gynostegium, and caudicles of the pollinaria with reticulated membranes and lateral teeth. Several systems of classification within Asclepiadoideae are highly artificial. Yet *Oxypetalinae* is the best circumscribed and characterized group within the subfamily. However, the infrageneric classification of *Oxypetalum*, remains problematic and with several artificial groupings being recognized. This project intends to redefine *Oxypetalum*, test the monophyly of subgenera within *Oxypetalum*, and study relationships among subgenera. In addition, the relationship between *Oxypetalum* and some of its close relatives, such as *Calostigma* and *Schistogyne* will also be investigated. FAPESP

P1010. Pollinator foraging behavior and effectiveness on sympatric *Asclepias* species

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Mechanical and ethological factors can provide pollinator-mediated floral isolation capable of reinforcing species barriers. We examined experimental and natural populations of three sympatric species of *Asclepias* for differences in pollinator effectiveness: *A. syriaca*, *A. incarnata* and *A. verticillata*. Previously we had detected significant variation in the frequency, constancy, and behavior of major pollinators on these species. Mechanically, pollinia attach differentially to the aroliar pad of insect legs in *A. syriaca*, but to tarsal hairs in the other species. Based on single visit removal and deposition of pollinia, as well as pollinium load on captured insects, we analyzed the relative effectiveness of insect visitors. Effectiveness fluctuated by species and by site. Major pollinators varied in their behavior for factors including the amount of time spent on umbels, number of flowers visited, and mode of contact with individual flowers. Overall, pre-mating barriers provide a potentially effective means of reducing interspecific pollen flow among plants visited by generalists.

P1011. Apocynacean floral ontogeny - gynoeceum development patternsS. M. Gomes¹, L. S. Kinoshita², M. d. M. Castro²;¹Pós-Graduação em Biologia Vegetal, Universidade Estadual de Campinas, Campinas, Brazil, ²Depto. de Botânica, Universidade Estadual de Campinas, Campinas, Brazil.

The floral ontogeny of 34 species of Apocynaceae was studied using SEM and results for five species are presented. The most relevant ontogenetic characteristics are: sepal initiation may be dextrorse, sinistrorse or both within the same inflorescence; congenital connation of the corolla tube basal region; reduction or absence of the corolla tube superior region; synchronous or asynchronous initiation of the petals and stamens; congenital adnation of the stamens with the corolla tube; postgenital adnation of the stamens with the style-head; appendicular origin of the nectaries. Four patterns of gynoeceum development are proposed: congenital syncarpy (*Lacmellea arborescens* Markgr., Rauvolfioideae), mixed syncarpy (*Allamanda blanchetii* A. DC., Rauvolfioideae), hemisyncarpy initiated as a concavity (*Malouetia arborea* Miers, Apocynoideae and *Ceropegia woodii* Schltr., Asclepiadoideae) and hemisyncarpy initiated as a dome (*Rhodocalyx rotundifolius* Müll. Arg., Apocynoideae). FAPESP, CNPq and LNLs.

P1012. The genus *Oxypetalum* R. Br. (Asclepiadoideae-Apocynaceae) in the state of Rio de Janeiro, BrazilN. M. F. da Silva¹, J. Fontella-Pereira², M. C. Valente¹;¹Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil, ²Museu Nacional, Rio de Janeiro, Brazil.

A synopsis of *Oxypetalum* R. Br. species from the state of Rio de Janeiro, Brazil, is presented, with an identification key, a short description of each species, illustrations, flowering and fruiting periods, and other observations. *Oxypetalum* R. Br. is a Neotropical genus with c. 131 species. The 25 taxa found in Rio de Janeiro are climbers with the exception of *O. patulum* E.Fourn., which is a sub-erect shrub. Most taxa occur in secondary Atlantic Forest and other are from sandy coastal plain (restinga) vegetation. Some taxa occur in high altitude grasslands, such as *O. costae* Occhioni and *O. patulum* E.Fourn. Some species are endemic to Rio de Janeiro, such as *O. lutescens* E.Fourn. and *O. schottii* E.Fourn. Two species are cited here as new records for the state of Rio de Janeiro: *O. molle* Hook. & Arn. and *O. pannosum* Decne. Some species are proposed as new synonyms, such as *O. sobralii* Occhioni and *O. mourai* Hoehne. *O. appendiculatum* Mart., *O. molle*, *O. pannosum*, *O. sublanatum*, *O. wightianum* Hook. & Arn., and *O. banksii* subsp. *banksii* are widely distributed, the first five reaching Paraguay and Argentina, while *O. banksii* subsp. *banksii* is limited to Brazil.

P1013. Phylogeographic investigation in *Biscutella* L. (Brassicaceae) based on morphological, genome size and molecular (ITS) dataC. König¹, A. N. Müllner²;¹Institute of Botany, Department of Systematic and Evolutionary Botany, University of Vienna, Vienna, Austria, ²Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey, United Kingdom.

The genus *Biscutella* comprises about 50 species, mainly found in Central and Southern Europe. Species delimitation is difficult, because most of the morphological differences are only quantitative. Several polyploidy series exist with tetraploids and hexaploids. In a cladistic analysis of 40 species 30 morphological characters were recorded and ITS 1 and 2 sequence variation was analysed. Phylogenetic trees show clear separation of the three main groups *londraba*, *Thlaspidium*, and *Laevigatae*, while resolution within the biggest group *Laevigatae* is quite low. *B. frutescens* and *B. megacarpa* are found at the basis of this clade, while *B. coronopifolia* and *B. laevigata* are in the most derived position. There can be observed a phylogeographic trend within the *Laevigatae* clade: phylogenetic development and spreading of the species seems to take place in a certain direction: from South-Western to Central Europe. This observation is also supported by flow cytometry measurements: genome size shows the lowest values of 0,78 pg (1C) in Southern Spain, increasing over 0,92 pg in Southern France up to finally 0.98 pg in the Southern Alps.

P1014. A preliminary investigation of phylogenetic relationships and species limits among diploid taxa of *Boechea* (Brassicaceae)L. Allphin¹, M. D. Windham², C. D. Bailey³, I. Al-Shehbaz⁴;¹Brigham Young University, Provo, UT, United States, ²Utah Museum Natural History, Salt Lake City, UT, United States, ³New Mexico State University, Las Cruces, NM, United States, ⁴Missouri Botanical Gardens, St. Louis, MO, United States.

Boechea was proposed by A. Löve and D. Löve (1976) as a North American segregate of cosmopolitan *Arabis*. Although separated by chromosome base number ($x = 7$, *Boechea* vs. $x = 8$, true *Arabis*), morphological distinctions are subtle and treatment of *Boechea* as a genus is not widely accepted. Recent studies using chloroplast and nuclear genes indicate that *Boechea* is phylogenetically isolated from *Arabis* and more closely related to *Halimolobos*, *Capsella*, and *Arabidopsis*. Consequently, most North American *Arabis* have been transferred into *Boechea*. We initiated detailed studies of this group and found a taxonomic complexity rarely encountered among higher plants. Molecular data indicate that *Boechea* appeared rather recently (< 3 mya). Subsequently, divergent speciation has produced >70 morphologically distinct, sexual diploids. Reproductive isolation has not kept pace with morphological divergence and diploid *Boechea* appear to hybridize in sympatry. Hybridization has given rise to a diverse array of facultatively apomictic diploids and obligately apomictic triploids and tetraploids, obscuring morphological distinctions among sexual diploids.

P1015. Intraspecific differentiation of *Arabidopsis halleri* based on AFLP and morphological dataM. Kolník¹, K. Marhold^{1,2};¹Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia, ²Department of Botany, Charles University, Prague, Czech Republic.

Three or four subspecies have been usually recognized in *Arabidopsis halleri*: E Asian subsp. *gemmifera*, C and SE European subsp. *halleri* and subsp. *ovirensis*, and sometimes also somewhat doubtful subsp. *tatica* from the W Carpathians. We studied 32 populations originating from the Carpathians and Alps in Europe, and from Japan, using morphometric and AFLP (amplified fragment length polymorphism) approach. Fifteen additional population samples were included in morphometrics only. As a result, four genetically well-defined groups can be delimited, corresponding to subsp. *gemmifera*, subsp. *tatica*, subsp. *ovirensis* from Austria, and populations assigned to subsp. *ovirensis* from Romania. Romanian populations, although previously treated within subsp. *ovirensis*, represent a clearly distinct lineage. Within subsp. *halleri*, which is on the basis of AFLP markers only weakly supported, genetic differentiation between the Carpathians and Alps can be observed. Morphometric analyses confirm morphological differentiation among the groups as resolved by AFLP data.

P1016. A floral homeotic variety of *Capsella*: evolutionary aspectsP. Nutt¹, B. Neuffer², G. Theißen¹;¹Genetics Department, University of Jena, Germany, ²Department of Systematic Botany, University of Osnabrück, Germany.

Homeotic mutants have been invaluable to understand plant development, but their role in evolution is controversial. To better understand the evolutionary significance of homeotic mutants we are studying a floral homeotic variety of *Capsella bursa-pastoris*. In the aberrant *Capsella* all petals are completely transformed into stamens. This variety is growing in several natural populations in the wild in Europe and Central Asia and it shows no obvious reduction in fitness. This 'Staminoid petals' (*Spe*) *Capsella* thus qualifies as a drastic morphological variant with the potential to establish a new evolutionary lineage. It hence serves as a suitable model to investigate the role of non-gradualistic structural changes during the evolution of the flower. We report the first results on the inheritance of the mutant phenotype and the isolation and phylogenetic evaluation of some candidate genes. Ongoing study efforts on the expression of candidate genes and on map based cloning are discussed.

P1017. Worldwide phylogeography of the genus *Draba* (Brassicaceae): Distribution patterns of genetic diversity.

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The genus *Draba* includes about 350 species, which are distributed primarily in the northern hemisphere with some 70 species in South and Central America, 115 species in N America, 45 species in China, 90 species in the former USSR, and 45 species in Europe. Many species are hardly distinguishable morphologically, and hybridization and reticulation is thought to have played a major role during its evolution and diversification. We studied DNA-sequence variation of the plastidic trnL-trnF-region to reconstruct the phylogeographic history of the genus *Draba*, which is distributed primarily in high-montane and alpine regions.

Our data strongly support hybridization and reticulation in this old genus and confirms Eurasian *Arabis* as closest sistergroup and herein we shortly present a generic circumscription.

First phylogeographic results favour the hypothesis of multiple immigration into North America from both amphi-beringean pleistocene refugias with subsequent hybridization between representatives from both areas. Further research is focused on Central and East Asian *Drabas* as one diversity center with taxa from elevations higher than 5500 m a.s.l.

P1018. Molecular basis and evolutionary consequences within the change in self-incompatibility system in the genus *Diplotaxis* (Brassicaceae)

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In the Brassicaceae, cross fertilisation is forced by a sporophytic self-incompatibility-system (SI), which has been intensively studied in the model plant *Brassica*. We focus on the diploid species pair *Diplotaxis tenuifolia*/*Diplotaxis cretacea*, which are closely related to *Brassica*. *D. tenuifolia* is strictly out-crossing, a successful coloniser and native to south and central Europe. The closely related *D. cretacea* is highly selfing, not colonising and locally restricted to the Northern Ukraine and adjacent parts of Russia. The distribution areas are separated by a gap of several hundred kilometres. The breakdown of the SI-system in *D. cretacea* might be correlated to its peripheral geographic position compared to the selfincompatible and widespread *D. tenuifolia*. The aim of the project is to reveal the molecular basis for the transition from SI to SC and characterise S-haplotypes in natural populations of both species mentioned.

P1019. Natural hybridization between *Cardamine raphanifolia* and *C. pratensis* (Brassicaceae) in NW Spain

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Sympatric occurrence of two polyploids, *Cardamine raphanifolia* Pourr. and *C. pratensis* L. (s. str.), was found on several localities in the Cordillera Cantabrica Mts. (NW Spain), together with morphologically intermediate individuals. Combined karyological, morphometric and molecular (AFLP, amplified fragment length polymorphism) approach was used to confirm assumed hybridization between these two species. Individuals from pure populations of both species were comparatively examined with individuals from putative hybrid populations. The results reveal extensive interspecific hybridization and introgression going on on several sites. Detailed sampling on one locality shows the role of habitat disturbance for successful hybrid establishment and persistence. Although hybridization between *C. pratensis* and *C. raphanifolia* does not seem to be rare, the type herbarium specimen of a previously described hybrid of these two taxa, *C. xlaramburgiana* Rouy et Foucaud, deposited in herbarium P, undoubtedly belongs to *C. raphanifolia*.

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P1020. The system for determination of pests and pathogens based on shown symptoms in cruciferous plants.

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The system is created for the model of the family *Brassicaceae* as an example of an important agricultural group of plants. In some constituents of this family there are described symptoms which are caused by diseases and pests. In this project the taxonomic revision of the family *Brassicaceae* was made. This system was

created using Php and MySQL software. Plants, pests and fungal pathogens are sorted according to Bayer's codes in the databases. The system contains taxonomical description in pests and pathogens, also their characteristics, bionomical data and exact definition of symptoms. The advantage of this system is the displaying of one particular symptom in particular plant caused by different pathogens. The system has a section for administrators where were inserted new plants, pests, diseases and types of symptoms. The relationships between new inserted data are created using sets of forms. In the whole system is used the correct scientific terminology with description of these terms therefore this system serves for the education and thanks to the used database MySQL it is easy to update it.

P1021. Phylogeny and biogeography of *Cardamine flexuosa* (Brassicaceae) and close relatives

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Based mainly on morphology, 10-15 species have been associated with two Eurasian species, tetraploid *Cardamine flexuosa* and diploid *C. hirsuta*. They include mostly autogamous annuals and biennials, with both diploids and polyploids represented. In the present study we aim to infer phylogenetic relationships within this group of taxa, using nuclear and chloroplast DNA sequence data. The results of both data sets are largely congruent and show several supported clades. Some of the main results can be summarized: 1) European *C. flexuosa* is different from populations in E Asia assigned to this species; E Asian populations represent a distinct lineage most likely of allopolyploid origin, as a weed invading Australia and America; 2) low genetic diversity is found in *C. flexuosa* and *C. hirsuta* across their large geographic area; 3) in contrast, E Asian tetraploids *C. scutata* and *C. niigatensis* show large haplotype diversity just within the small area of Japan; 4) N American diploid *C. oligosperma* often confused with *C. hirsuta* is distinct from the latter; 5) polyploids and diploids are separated in distinct clades, thus the origin of most polyploids remains unclear.

P1022. Molecular phylogeny of the tribe Brassiceae (Brassicaceae)

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Evolutionary relationships of the tribe Brassiceae (Brassicaceae) were examined using sequence data from the ITS region and *trnL* intron and cpDNA restriction site polymorphism data. The tribe (ca. 50 genera) is distinguished by the presence of conduplicate cotyledons and/or heteroarthrocarpic fruit. cpDNA restriction site data and ITS and *trnL* sequence data were obtained for 22, 104, and 94 taxa in the tribe, respectively. Maximum parsimony analyses of the cpDNA and ITS sequence data showed a monophyletic origin for the tribe, with the inclusion of controversial members *Calepina*, *Conringia* and *Orychophragmus*. Molecular ITS clades corresponded to taxonomic subtribes: Vellinae, Zillinae, and Savignyninae; but as with cpDNA data, there was little support for subtribes Brassicinae, Raphaninae and Moricandiinae. Taxa from these three subtribes were previously assigned to either the Rapa/Oleracea or Nigra cpDNA lineages. Although there was no support for these two lineages, many cpDNA subclades within each of them were evident in the ITS-based phylogeny. The *trnL* data provided no resolution of tribal limits or relationships within the tribe.

P1023. Molecular Systematics of the Central Asian genus *Dontostemon* (Brassicaceae)

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The genus *Dontostemon* is a Central and East Asian genus distributed primarily in Mongolia, Russia and China and comprising twelve species. *Dontostemon* species prevail in arid regions, mainly steppe, desert and mountain habitats. Genetic relationships

within *Dontostemon* and phylogenetic position of the genus within the family Brassicaceae are unknown. We started a molecular analysis (ITS; *trnL* intron and *trnL-F* spacer) to trace the phylogenetic roots of this characteristic Central Asian - Far East floristic element. Data argue for a close relationship between *Dontostemon* and *Clausia* which formed a monophyletic clade. It would appear that *Matthiola* is closer related to this clade than *Hesperis*. Phylogenetic relationships between the *Dontostemon* species are not clearly resolved. However, three well supported clades are obvious: An eastern Asian clade, a clade with species from South Siberia, Mongolia and northern China, and a clade with species from western Mongolia and northern China.

P1024. Phylogenetic relationships of Brassicaceae genera from Northern Asia

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Central Asia and the Russian Far East experienced a paleoenvironmental history different from that in Europe and in North America. The loss of biodiversity during the Ice Ages has been less dramatic than in Europe. Consequently, Siberia is of high biogeographic significance for the postglacial history of the European flora as is the Far East for North America. About 70 genera of Brassicaceae are reported from northern Asia, a considerable amount of which being endemic to this region. Two thirds are mono- to oligotypic genera, and only ca. 20 genera comprise three or more species. This taxonomic situation and the paleoenvironmental history let us to assume that some of the present day Brassicaceae in northern Asia represent preglacial elements, and others may have evolved recently in postglacial times. We study molecular phylogenies (ITS and non-coding cpDNA) to elucidate phylogenetic relationships, origin and age of northern Asian Brassicaceae taxa.

P1025. The role of lignification patterns in dehiscent and indehiscent fruits in Brassicaceae: a comparative anatomical approach

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Controlling seed dispersal remains an important challenge for growers of many agricultural crops worldwide. Scientists are beginning to understand the regulatory networks underlying the dehiscence (opening) of fruits. In *Arabidopsis* (Brassicaceae) only a few genes seem to control fruit dehiscence and promote the lignification of fruit valve margin cells. To evaluate the impact of lignification on the dehiscence process, we have examined the lignification patterns of valve margin cells in both dehiscent and indehiscent fruits of wild Brassicaceae species. Our first results indicate that in dehiscent fruits a strip of 1-2 longitudinal rows (parallel to the septum) of unligified cells is left at each side of the replum (a thin structure that separates the fruit valves), functioning as a layer separating the valves from the replum, i.e. the dehiscence zone. In the indehiscent fruits lignification of parenchymatous tissue is uninterrupted and, therefore, no dehiscence zones are formed.

P1026. Darwin's finches with four petals: Diverse endemic Capeverdean *Diplotaxis* (Brassicaceae) are monophyletic and radiated recently

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It has recently been postulated that the Capeverdean flora including its endemic elements is in contrast to previous beliefs very young, probably not older than a few hundred thousand years. To test this hypothesis we are studying molecular phylogenetics (ITS and non coding cpDNA) associated with a molecular clock approach analyzing representatives of *Diplotaxis* and allies (Brassicaceae). Nine *Diplotaxis* taxa, characterized by a very high level of morphological diversification, are endemic to the Macaronesian Cape Verde Island. Our data provide evidence for monophyly of the endemic Capeverdean species and a North African origin. DNA sequence differences among the Capeverdean species indicate diversification of the endemic Capeverdean taxa in Quaternary times, 0.6 to 1.2 million years ago. This adds to the emerging pattern that the present Macaronesian flora is of quite recent origin.

P1027. Rare *Camelina* weeds (Brassicaceae) in Slovakia

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The genus *Camelina* included four annual or winter annual weedy species in Slovakia: *C. alyssum*, *C. microcarpa*, *C. rumelica* and *C. sativa*. *C. microcarpa* is relatively widespread, other three species were classified as critically endangered (*C. rumelica* and *C. sativa*) or extinct species (*C. alyssum*). Two subspecies of *C. alyssum* were found - subsp. *alyssum* and subsp. *integerrima*. Both taxa were already rare in the past; we recorded only 22 and 12 localities, respectively. Only 10 localities of *C. rumelica* were recorded at all, some of them were not native. Two new localities near Kamenica nad Hronom and Bajtava (southwestern Slovakia) were found in our field research. Herbarium samples of *C. sativa* involved two taxa - subsp. *sativa* (11 localities) and subsp. *zingerii* (5 localities). The most recent data of distribution of subsp. *zingerii* on southern Slovakia, Chlaba (1999) and Hajnácka (2001), were not confirmed in our study. Subsp. *sativa* is still infrequently cultivated.

P1028. Breakdown of self-incompatibility and speciation in *Capsella* (Brassicaceae)

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The transition from a self-incompatible (SI) to a self-compatible (SC) mating system is a widespread phenomenon in the Brassicaceae and is often associated with colonising success. The breakdown of the SI system might be a key event in evolutionary processes in the Brassicaceae. Aim of the project is to uncover the molecular basis for the switch from SI to SC, moving out from the knowledge in the model plant *Arabidopsis* to wild genera in the Brassicaceae. Focus is on the diploid taxa *Capsella grandiflora*, a non-colonising outbreeder endemic to the western balkan and on *C. rubella*, an inbreeding worldwide coloniser of mediterranean climates. We identified S-genes, tested for dominance of SC over SI and for linkage of S-genes, so far. Additional screening of a BAC library (coll. J. Kroymann) will unmask complete genes. Subsequent comparative analysis of the S-genes in wild populations of SI and SC taxa in *Capsella* will help to understand the genetic background of the breakdown of SI.

P1029. Phylogeography of *Schivereckia podolica* (Brassicaceae) and Ice Ages in East Europe

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Schivereckia podolica is endemic to eastern Europe where it is distributed from the Ukraine in the West to the Ural Mts. in the East. It is confined to rocky outcrops and has a disjunctive distribution area. RAPD data argue that this disjunction is the result of fragmentation of a formerly continuous distribution belt which is interpreted as a subarctic steppe along the periglacial environments of the last glacial period. DNA sequence divergence and RAPD data together with the present distribution of *Schivereckia podolica* argue for a recent origin of the disjunction, between 300,000 and 150,000 years BP (middle Pleistocene, Oka glaciation, to young Pleistocene, Dnjepr glaciation). Molecular analyses also revealed that the genus *Schivereckia* (two species are currently recognised) is monophyletic and nested within *Draba*, some arctic/subarctic and/or alpine *Draba* species forming a sister clade. This supports the interpretation of *Schivereckia podolica* as a glacial relic.

P1030. A Revision of the Genus *Isatis* (Brassicaceae) in Iran

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Although widely spread in Irano-Turanian region, the extremely difficult genus *Isatis* L. has not been adequately covered by floristic and other studies, mainly due to exceptional variability of the species, inadequate material and illustrations, and lack of cross-cutting research.

The present paper attempts to revise this genus in Iran with a combination of morphologic and anatomic studies. The available specimen of the 14 reported species in the major herbaria and the fresh samples gathered from various provinces were examined in

the light of the major flora of the region and the microscopic images of the anatomy of stem, leaves and fruits.

As a result, a revised description of the taxons and their geographic distribution and a new key to the genus in Iran were prepared. Three new records were made for Iran, and the status of some taxons was revised.

Adequate morphologic (including whole plant, fruits and characteristics leaves) and anatomic (stems, leaves and fruits) images and illustrations of all species were prepared.

P1031. Inflorescence architecture of Brazilian species of *Aechmea* subg. *Chevaliera* (Gaudich. ex Beer) Baker, Bromelioideae - Bromeliaceae

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Inflorescences in Bromeliaceae are indeterminate, supported by a terminal scape of various size, and have bracts of various position, number, color, shape and texture. *Aechmea* is the most diverse genus of Bromelioideae (ca.220 spp) presenting large inflorescence and variation in size and shape. *Aechmea* subg. *Chevaliera* comprises 21 taxa throughout Central and South America mainly distributed in eastern Brazil. A morphological inflorescence study of 15 Brazilian species is presented. The study was based on living specimens and herbarium materials with the inflorescence architecture observed at different developmental stages. This study describes the architectural form of the inflorescences at full-flower or with at least half of the flowers open, because fruit development may produce architectural changes in the axis. The inflorescence in the subgenus ranges from simple to compound. Spicate inflorescence is the basic pattern, and capitulum is here recorded for the first time in Bromeliaceae. Compound inflorescences are the rarest form in the subgenus, being represented by racemes of spikes. Descriptions, illustrations, and schematics are presented.

P1032. Phylogenetic inference involving some Bromelioideae genera, using *rps16* and *atpB-rbcL* sequences and morphology.

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Among the three subfamilies of Bromeliaceae, Bromelioideae (ca. 30 genera) is the poorest known. *Portea* Brongn. ex K. Koch, a Bromelioideae genus endemic to Brazil, comprises nine species distributed in Northeast and Southeast Brazil. Usually, it is regarded as closely related to *Aechmea*. A phylogenetic analysis was carried out based on morphology (gross morphology, leaf-anatomy, palinology) and cpDNA sequences (*rps16*, *atpB-rbcL*). *Quesnelia arvensis* was used as outgroup and the analysis involved three species of *Aechmea* (*A. bromeliifolia*, *A. disticantha*, *A. nudicaulis*), two of *Canistrum* (*C. aurantiacum*, *C. pickelii*) and seven of *Portea* (*P. alatisepala*, *P. grandiflora*, *P. kermesina*, *P. leptantha*, *P. nana*, *P. petropolitana*, *P. silveirae*). Parsimony analysis of the combined data indicates that 1) *Portea* is monophyletic, 2) *Canistrum* is paraphyletic, 3) *Canistrum* is closely related to *Portea* (bootstrap value 98), and 4) *Aechmea* is polyphyletic.

P1033. The Effect of Speciation and Extinction Rates on Phylogenetic Branch Lengths in Bromeliaceae subfamily Bromelioideae

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Members of Bromeliaceae subfamily Bromelioideae exhibit their greatest diversity and are considered keystone species within the Atlantic Forest region of eastern Brazil. The Atlantic Forest has been subject to extensive reduction and fragmentation and is recognized as one of the most endangered biomes on the planet. Phylogenetic analyses of molecular and morphological data in Bromelioideae have consistently yielded a pattern in which terminal branches of the phylogeny are significantly longer than internal branches. Two hypotheses are proposed to explain this

discrepancy in branch lengths: 1) members of Bromelioideae have recently derived stronger isolating mechanisms among closely related species than they possessed during earlier periods in their evolution; 2) the subfamily has recently experienced an increase in extinction rate that is not accompanied by a comparable increase in speciation rate. The latter hypothesis is consistent with simulation studies of variable speciation/extinction rates, and the elevated extinction rate is hypothesized to be a direct result of extensive habitat loss due to destruction of the Atlantic Forests of Brazil.

P1034. Towards a molecular population genetics of four *Alcantarea* species (Bromeliaceae) adapted to inselbergs in the Atlantic Rain Forest of Brazil

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The inselbergs of the Brazilian Atlantic Rain Forest form terrestrial "islands" that are isolated from the surrounding forest by steep gradients in humidity, temperature and irradiation, and by drastic changes in soil substrates. Bromeliads (Bromeliaceae) are particularly well represented among plants adapted to the extreme growing conditions on inselbergs. We have launched a research project to study the population genetics of four members of the genus *Alcantarea* endemic to inselbergs of coastal Atlantic Brazil, *Alcantarea imperialis*, *A. geniculata*, *A. glaziouana*, and *A. regina*. Our aim is a comparative study of genetic variability at nuclear DNA microsatellite and plastid DNA markers. We address fundamental questions regarding the evolution of genetic diversity within and among these four species. First results of this work will be presented, including a set of novel polymorphic microsatellites for *Alcantarea* spp.

P1035. Phylogenetic relationships of *Sodiroa* (*Guzmania*, Bromeliaceae) based on morphological and molecular data

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Sodiroa s.l. (Bromeliaceae) is a group of species traditionally included in *Guzmania*, and made up of two species complexes: *Massangea* and *Sodiroa* s.s.. Previously considered separate genera, *Sodiroa* s.s. comprises 7 species and *Massangea* 11. The species are found from south Central America to northern Peru, with the greatest diversity occurring in the Pacific region of Colombia. Preliminary analyses of morphological data suggest that *Sodiroa* s.l. is a monophyletic group, whereas *Massangea* is paraphyletic. We sequenced DNA from 16 species of Bromeliaceae, in order to test this hypothesis, using separate and combined analyses of chloroplast genes (*matK*, *ndhF*, *trnL* intron, *trnL-trnF* intergenic spacer) and morphological characters. Additional sequences were taken from GenBank. Individual analyses show that *trnL-trnF* provides the best resolution, and *Sodiroa* s.l. is monophyletic. The combined molecular analysis recovers a monophyletic group that includes *Sodiroa* s.l. and two other *Guzmania* species. We intend to code additional morphological characters for all the species in order to initiate a total evidence analysis for this group of Bromeliaceae.

P1036. Molecular systematics of *Fosterella* (Bromeliaceae)

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The majority of species of the neotropical genus *Fosterella* (Bromeliaceae) are found in semiarid to humid habitats of the Andean slopes and valleys of Bolivia. Numerous new taxa have been described in the past decade, raising the number of known *Fosterella* species to about 30. *Fosterella* plants are mesophytic terrestrial herbs with small and inconspicuous flowers. Morphological differentiation of species is difficult due to a paucity of characters, and little is known about the intrageneric phylogeny. Traditionally, *Fosterella* is included in the subfamily Pitcairnioideae, but recent molecular data have challenged this classification. Whereas the monophyly of the genus is generally accepted, sister group relationships of *Fosterella* within Bromeliaceae are ambiguous. To analyze the phylogeny of

Fosterella at the species and genus level, we have initiated a molecular phylogenetic study based on AFLP markers and comparative sequencing of non-coding chloroplast DNA. Here we present preliminary results.

P1037. Phylogenetic analysis of *Vriesea* (Bromeliaceae) based on morphological data

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The tillandsioid genus *Vriesea* includes 230 species, in two sections - *Vriesea* (100 spp.) and *Xiphion* (130 spp.). In this study, a partial cladistic analysis of the genus was done based on morphological data to evaluate the relationships within several groups of species and trace the phylogenetic relationship of the sections. Sixty eight taxa were selected for the ingroup, two species of *Catopsis* as outgroup; and 68 characters. Three hundred and sixty four equally parsimonious trees were produced in 734 steps (CI 0.219, RI 0.576 and RC 0.126). Two major clades were formed based on type of inflorescence branching. The two sections emerged as polyphyletic. However, several other clades emerged made up of taxa of one or the other of the sections. Some of the characters which supported the clades are used traditionally to separate sections. Other characters emerged as new traits, potentially important in genus taxonomy. Future analyses should include as many representatives of the tillandsioid group as possible to sustain a new interpretation of the subfamily Tillandsioideae.

P1038. Stem and leaf anatomy of Bromeliaceae from the cerrado of São Paulo State, Brazil

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The Brazilian cerrado vegetation (savanna) is one of the most unique biomes, covering around 23% of the country and comprising a diverse flora with over 6,000 species. Despite its importance, agriculture has been developed in the cerrado causing massive destruction. In the cerrado of São Paulo State there are 15 terrestrial and epiphytic species of Bromeliaceae. In these plants, the rhizomes and the floral scape have a parenchymatous cortex that in some species is limited from the central cylinder by a ring of sclerified cells (pericycle). Intracortical roots and leaf traces are commonly found in the cortex of the rhizome. The leaves are hypostomatic and bear peculiar stalked scales and silica bodies on their epidermis. Some features of the leaves, such as the presence of adaxial palisade parenchyma, extra-vascular fibers and/or secretory cavities in the mesophyll, are restricted to particular species. In addition, the location of water storage tissue, the nature of the thickness of the epidermal cells, the presence or absence of a mechanical hypodermis and the stomata position, vary between species and may characterize them.

P1039. Leaf anatomy of *Fosterella* (Bromeliaceae)

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The genus *Fosterella* comprises 30 species, principally distributed in arid and semihumid habitats and extending from Mexico to Argentina. Its center of diversity is located in the Andes of Bolivia. Some species are morphologically quite similar and their delimitation remains problematic.

The leaf anatomy of 24 species and 41 accessions of the genus was studied. Transverse sections of the leaf-blade were made from unstained fresh material by hand. Microtome sections were obtained from material fixed in FAA and embedded in HEMA, and stained with toluidine. The analysis included digital measurements, e.g. of tissue areas in comparison to the whole section.

Several characters, especially related to the epidermis, chlorenchyma and the position of vascular bundles proved to be of taxonomic value. Groups of species characterised by similar leaf anatomy are presented and compared to groups based on molecular data. Abstract will be improved to get to the minimum number of characters.

P1040. Water storage tissue patterns in the leaves of Bromeliaceae

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Almost all the species of Bromeliaceae have succulent features, one of the most important is water storage tissue. The study of 9 species which belong to 3 subfamilies and 4 life forms (terrestrial xerophyte and mesophyte, tank and aerial epiphyte) showed different patterns of water storage tissue. The most massive water storage tissue have terrestrial xerophytes and aerial epiphytes, tank epiphytes and mesophytes have thin layers of this tissue. We recognised 3 different groups in investigated species that are based on water storage tissue patterns. The first group is with the layer of chlorenchyma under the layer of water storage tissue. The second one has 2 layers of water tissue and a layer of chlorenchyma between them. The third group without abruptly differentiated tissue layers: chlorophyll cells organised in the net with water storage cells inside. These 3 groups mark 3 trends of evolution and analysis of anatomy allows to suppose that the first pattern is the most primitive.

P1041. Circumscribing *Vellozia hirsuta* and *V. tubiflora* (Velloziaceae)

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Vellozia hirsuta and *V. tubiflora* belonged to *Vellozia* sect. *Radia*, a poliphyletic taxon, although *V. hirsuta* and *V. tubiflora* emerge as close related in some analyses. From the 21 species admitted in the last revision of the section, I only recognise three. This reflects different choices of characters for delimiting the species. Authors choosed the indument, the dimensions of the pedicel and of the hypanthium tube to distinguish species within the section, but these characters may vary within species and populations. Better characters distinguishing them are that also used in cladistic analyses. *V. hirsuta* is circumscribed by one phloem strand and solitary flowers; *V. tubiflora* by smooth subsidiary cells in stomata, aquiferous hypodermis extending to bundle sheaths and furrows, vanishing integument of empty cells in seeds, and a sclerified belt in pedicels. The hypanthial tube always longer than ovary, and deciduous in fruit, distinguishes *V. hirsuta* and *V. tubiflora* from all other Velloziaceae. Although consistently delimited, both species exhibit wide intraspecific variation. Thus, *V. hirsuta* encompasses four taxonomic synonyms, and *V. tubiflora*, 19.

P1042. Revision of *Hohenbergia* Schult. & Schult.f. subgenus *Hohenbergia* (Bromeliaceae)

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Hohenbergia Schult. & Schult.f. subg. *Hohenbergia* is a neotropical group of bromeliads found mainly in altitudes from sea level to about 800 m elev. The mains morphological characters distinguishing this group are the 3-4-pinnate inflorescences, peduncled spikes, purple, green or yellow corollas, and caudate ovules. The subgenus is treated based on a morphological study of field-collected material and herbarium specimens. Forty-five epithets have been applied to the species of these group, and fifteen are recognized as synonyms. In this work the subgenus *Hohenbergia* consists of 30 species and occurs from Trinidad and Tobago to Venezuela and east coast of Brazil. All species are found in Brazilian territory, including *H. stellata* Schult. & Schult.f., the unique species with disjunct distribution. The principal center of diversity of the subgenus is the Brazilian State of Bahia with 28 species recorded (93% of the total). Of these, 23 species (77%) are endemic and found from Center-South to South of Bahia distributed through semiarid areas locally denominated "Caatinga" to remaining coastal rainforests in seaside direction. Sponsored by CAPES, IMSEAR-CNPq.

P1043. Phylogenetic analysis of the tribe Salsoleae (Chenopodiaceae) using ITS rDNA sequencing in connection with systematics and reconstruction of morphological evolution character

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The tribe Salsoleae includes approximately 30 genera distributed in ancient Mediterranean region. Regrettably, almost all morphological features used in tribe Salsoleae taxonomy do not reflect their phylogenetic relationships and can be probably a result of genetic assimilation of similar ecological modifications. A revision of morphological features taxonomical value for tribe Salsoleae systematics is necessary as well as search of new significant characters. The comparison of morphological and molecular features will allow to evaluate the significance of the former. We report a preliminary analysis of the phylogenetic relationships among Salsoleae genera using ITS 1-2 rDNA sequencing. The distribution of tribe Salsoleae genera contradicts to standard subdivision of the tribe into subtribes and genera. ITS phylogeny supports a sister-group relationship between Climacoptera and Halimocnemis, Ofaiston and Petrosimonia (100% bootstrap support). The possibility of tree-like life forms and aphyllous independent occurrence in different taxa is discussed in connection with aridity of the climate. The project is supported by RFBR grant 04-04-49010.

P1044. Taxonomical importance of testa micromorphology in the American taxa of *Sarcocornia* A.J. Scott (Chenopodiaceae)

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Sarcocornia A. J. Scott (Chenopodiaceae) includes shrubs succulent, erect to prostrate and sometimes creeping, with seeds smooth or hairy and lacking perisperm. Other morphological characters such as size, habit, succulency, inflorescence, etc., are strongly convergent and taxa identification is usually difficult. In the Americas up to 20 taxa have been described based on weak morphological vegetative differences. Moreover, different nomenclatural treatments are available from different authors, as taxa of *Sarcocornia* have not yet been studied as a whole in the Americas. Seed testa micromorphology has revealed however as an important character to differentiate taxonomic entities in that genus. In the present contribution, the taxonomical importance of testa indument is discussed, and a morphological characterization of the accepted American taxa is reported.

P1045. Morphology and practical identification of *Chenopodiaceae* pollen (taxa occurring in Ukraine)

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Identification of *Chenopodiaceae* pollen below the family level was often considered impossible or highly unreliable. We present here the results of integral studies (using optical and scanning electron microscopy) of pollen morphology of more than 80 species of *Chenopodiaceae* occurring in Ukraine and adjacent areas. The main objective of the study was to develop reliable identification approaches and tools (keys and descriptions) to be used for taxonomy and pollen analysis. We propose a new identification manual for fossil pollen of *Chenopodiaceae*, which is based on our data and the principle of character correlation (shape and outline, pollen grain and pore diameter, distance between pores, number of pores, exine peculiarities). For the first time we propose to use for identification some basic qualitative characters (sculpture and texture of the exine). The problems and tasks of species-level and group-level identification of *Chenopodiaceae* pollen in pollen spectra of the Pleistocene and Holocene of Ukraine are considered. Palynological peculiarities and traits of selected natural phylogenetic groups of *Chenopodiaceae* are discussed.

P1046. Phylogenetic analysis of the tribe *Salicornieae* based on *matK* and *trnL-trnF* chloroplast DNA sequences

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The systematic and phylogenetic relationships between the annual genus *Salicornia* and the allied perennial genera of *Salicornieae* (*Sarcocornia*, *Arthrocnemum*, *Halocnemum*) were investigated by applying a molecular approach.

Two plastidial markers, the *matK* gene and the non-coding *trnL-trnF* interspacer, were sequenced from 40 samples belonging to

12 species and representing 4 genera of the tribe *Salicornieae*. The same sequences from *Spinacia oleracea* L. (subfamily *Chenopodioideae*) and *Suaeda maritima* (L.) Dumort. (subfamily *Suaedoideae*) were used as outgroups.

Samples were collected in different sites of Mediterranean and Atlantic European coasts and identified according to standard analytical keys.

Phylogenetic analyses were performed using PAUP 4.0, according to maximum likelihood and maximum parsimony methods.

Our main results strongly favour: 1) a monophyletic genus *Salicornia*; 2) a clear separation between the annual and perennial genera, a finding supported also by a peculiar six-bases insertion in the *matK* gene of the annual forms; 3) a sister group relationships between the genus *Sarcocornia* and the genus *Salicornia*.

P1047. The *Chenopodiaceae* of Armenia- taxonomical investigation

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The *Chenopodiaceae* are represented in Armenia with 89 species of 32 genera. All these genera are known from the Caucasian flora, except *Microcnemum*, present in Armenia. The complex genera in our area includes the following sections: Beta (4 species) - *Vulgares*, *Corollinae*; *Chenopodium* (14 species) - *Ambrina* (adv.), *Botryoidea*, *Eublittum*, *Degenia*, *Pseudoblittum*; *Atriplex* (11 species) - *Dichrosperma*, *Teutliopsis*, *Sclerocalymma*, *Obione*; *Kochia* (3 species) - *Pterocarpus*, *Semibassia*; *Suaeda* (7 species) - *Schanginia*, *Lachnostigma*, *Conosperma*, *Heterosperma*; *Salsola* (14 species) - *Cocosalsola*, *Salsola*, *Caroxylon*, *Malpighipila*, *Belanthera*, *Physurus*; *Anabasis* (2 species) - *Adenophora*, *Euanabasis*. Habitats and distribution - arid, salty, clayey, sandy and rocky areas, from 700 up to 1200 (2000) m, for the most part in the Yerevan and Meghri floristic regions of Armenia.

P1048. Diversification in *Camphorosmeae* (Chenopodiaceae)

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Camphorosmeae are a monophyletic tribe of *Salsoloideae* comprising subshrubs or shrubs that mainly occur in semi-deserts, deserts, and steppes. Molecular data show three major lineages within the tribe: 1. C Asia/Australia (13 gen., c. 150 spp.), 2. Eurasia/S Africa (3-9/c. 30), and 3. a lineage of five widely disjunct species (N Am., S Afr., Eurasia). After their origin in Eurasia in the Oligocene, the three lineages evolved along different tracks: The C Asian/Australian lineage entered Australia in the late Miocene where it experienced a steep increase in diversification rate in the newly forming arid landscapes of W and C Australia. In contrast to this, no shift of diversification rate took place in the Eurasian/S African lineage although this lineage evolved C4 photosynthesis with a high diversity of C4-leaves. We explore the possibility that this difference is due to stronger competition by other equally well-adapted steppe and semi-desert inhabitants in Eurasia and S Africa. The high genetic and leaf anatomical diversity of the five species of the third lineage suggests that these are remnants of old *Camphorosmean* lineages that have largely gone extinct.

P1049. Reproductive isolation between sympatric species of the northern European *Salicornia* (Chenopodiaceae) species complex

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Salicornia is notorious for taxonomic difficulties which have been explained with paucity of morphological characters, high phenotypic plasticity, frequent hybridization, and the inadequacy of dried material. Molecular studies based on sequence data and AFLPs, however, have shown that distinct clades can be recognized within the genus, and that substantial AFLP variation can be found among sympatric morphotypes/species.

Along the coast of northern Germany two diploid and two tetraploid morphotypes of *Salicornia* occur in close proximity. Although intermediate forms are known these morphotypes are stable and probably reproductively isolated. To show that the morphotypes observed have a genetic basis, and to understand the prevalent mechanisms of their reproductive isolation, we 1. study their reproductive biology and breeding systems and 2. try to find

matching AFLP genotypes. This detailed local analysis is supplemented with samples from the Atlantic coast of France and the English coast to obtain an idea of the distribution of morpho/genotypes along the northern European coasts.

P1050. The flora of steppes and deserts of the South-East of European Russia: the current research state.

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The South-East of European Russia (Astrahan, Volgograd, Saratov regions, Kalmykia) are characterized by the arid climate, the absence of mountains, unique salt lakes and sand desert Naryn.

The researched flora consists of 952 species, belonged to 337 genera and 59 families. The leading positions are occupied by species Asteraceae, Fabaceae, Poaceae, Chenopodiaceae, Brassicaceae, Caryophyllaceae, Liliaceae, and some genera Astragalus, Allium, Artemisia, Euphorbia. Among them the leading positions belongs to the species of Mediterranean geoelements. About 60% percent of the flora have near the verges of their areas. It is explained by its presence in the contact area of different climate zones. The presence of endemic kernel is its specific feature though it is not a characteristic of the plain flora. It can be explained by the complex geological history of the region. The transgressions of the Caspian Sea and intense glaciation in the North in Pliocene and Pleistocene guaranteed the migrations and the isolation of the species, the formation of endemics.

P1051. Cytogenetic characterization of *Chenopodium quinoa* genom

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Molecular cytogenetic analysis of *Chenopodium quinoa* was performed using fluorescent *in situ* hybridization (FISH) and flow cytometry. This Andean pseudocereal possess $2n=4x=36$ very small chromosomes and its genome size was determined as 3,01 pg /2C. The sequences included in FISH analysis derived from conserved plant repetitive sequences (25S rDNA and 5S rDNA, telomeric) and isolated from *C. quinoa* genome disperse repeats. Double FISH with 25S rDNA and 5S rDNA revealed that these sequences are located in a very few loci and allowed to distinguish only three homologue chromosome pairs. Hybridization signals of telomeric repeats were observed exclusively on chromosome termini.

Retroelement-like, transposon-like sequences and disperse repetitive sequences without homology to mobile elements were detected as relatively weak, but discrete hybridization signals on every chromosome. In cases of mobile elements hybridization signals were preferentially localized in a pericentromeric region whereas loci of disperse sequence without homology to mobile elements were distributed throughout all chromosomes.

P1052. Polyploidy in subgenus *Brezia* (Moq.) Freitag et Schütze of the genus *Suaeda* (Chenopodiaceae)

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The subgenus *Brezia* includes about 30 species. The chromosome numbers of 16 species (including undescribed taxa) have been studied from 150 natural localities mainly in East Europe and North Asia. As the intrageneric taxonomy of the subgenus *Brezia* has not been established yet, the most records are listed according to the subclades separated by Schütze et al. (2003) on the basis of molecular data. **1** - "prostrata group": diploids ($2n=2x=18$) *Suaeda prostrata* Pall., *S. olufsenii* Pauls., *S. heterophylla* (Kar. et Kir.) Bunge, *S. stellatiflora* G.L. Chu, *S. "orientalis"*. **2** - "maritima group": diploids *S. crassifolia* Pall., *S. heteroptera* Kitag.; tetraploids ($2n=4x=36$) *S. salsa* Pall., *S. macrocarpa* Moq. **3** - "corniculata group": diploid *S. tschujensis* Lomon. et Freitag, tetraploid *S. "grubovii"*; hexaploids ($2n=6x=54$) *S. corniculata* (C.A.Mey.)Bunge, *S. patagonica* Speg. Octoploids ($2n(8x)=72$) *S. "sibirica"*, *S. "ircutensis"* and decaploid cytotype ($2n=10x=90$) of *S. "sibirica"* hold a separate position which is in need of further consideration.

P1053. Inflorescence and flower development and taxonomy of the genus *Salicornia* (Chenopodiaceae) in European Russia

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Main inflorescence of most *Salicornia* species is a spike of 3-flowered dichasia. Dichasia are situated in axils of reduced opposite leaves. Flowers have 3-4 almost completely united tepals, (1)2 stamens in median position, and a gynoeceum with single ovule. In *Salicornia heterantha* Pankova & Demina, sp. nova, perianth tube of the central flower of dichasium is completely postgenitally fused to main axis of the spike. Perianth tubes of lateral flowers are free. In other Russian species all flowers are free. Early developmental stages are similar in all species studied. Central flower of dichasium is initiated first, followed by two lateral flowers. No lateral flower subtending bracts were found. Tepals are initiated almost simultaneously as separate primordia. They fuse congenitally later. Adaxial stamen is initiated before abaxial one. Gynoeceum is initiated as a ring-primordium. Ovule occupies a central position in the flower; its primordium is well visible before gynoeceum closure. Our data on reproductive morphology and development helps to clarify taxonomy of Russian *Salicornia*.

P1054. Combined analysis of the subfamily Gomphrenoideae (Amaranthaceae) using *rpl16* and *trnL-F* data

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The Amaranthaceae (70 genera and ~800 species) occurs in tropical and subtropical regions and it is commonly classified into two subfamilies. Subfamily Gomphrenoideae has bisporangiate anthers, whereas anthers in Amaranthoideae are tetrasporangiate. The chiefly neotropical Gomphrenoideae contains 19 genera and 400 species. In order to test monophyly and generic relationships of the Gomphrenoideae, this study included a larger sampling of the subfamily and applied different molecular markers from previous studies. A combined analysis using *trnL-F* and *rpl16* indicated that Gomphrenoideae is monophyletic. There are three major clades and none of these support previous generic classifications. The first clade includes *Iresine*, *Irenella*, and *Woehleria*, a relationship that is also supported by pollen data. A second clade contains *Pseudoplantago* (traditionally treated as the sole genus in the tribe Psuedoplantageae), *Gomphrena*, *Pfiaffia*, *Hebanthe*, *Xerosiphon*, *Froelichia*, *Guilleminea*, *Gossypianthus*, and *Blutaparon*. The last clade includes *Tidestromia* as the sister group to a weakly supported *Pedersenia* and *Alternanthera* clade. These results indicate the need for a new tribal classification since currently accepted tribes and subtribes do not reflect natural groups.

P1055. C3 and C4 Chenopodiaceae species in the vegetation of the Urals: their comparative characteristics and distribution in relation to soil type and climate

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The Urals is situated on the Europe-Asia border and stretches from steppes to tundra that fits this region for comparative ecology investigations. The list of Chenopodiaceae species of the Ural flora is made using literature data and herbarium material and it currently contains 88 species from 24 genera. 21 listed species from 8 genera of subfamily Salsoloideae and 11 species from 4 genera of subfamily Chenopodioideae possess C₄ photosynthesis. Species richness decreases northwards from 81 species in the regions close to Russia-Kazakhstan border to 12 ones in the North Urals. The only C₄ species reaches the tundra zone - *Atriplex tatarica* L. - was collected there on railways. The most part of the C₄ Chenopodiaceae species (81%) is a component of the native vegetation of arid and salt areas of the South Urals, the part of native C₃ species is less (57%). Annuals dominate both among C₃ (91%) and C₄ (72%) species. Almost all C₄ chenopods are succulents, whereas only about 20% of the C₃ ones possess this trait. The distribution of taxonomical and physiological (C₃ and C₄) groups in relation to precipitation, temperature, aridity and soil type is discussed.

P1056. Phylogenetic relationships in *Blumea* (Asteraceae: Inuleae)

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The genus *Blumea* (Asteraceae: Inuleae) comprises around 100 species distributed in tropical Asia, with some species in Australia and Africa. The delimitation of the genus has varied, and some species placed in *Blumea* have even been transferred to genera of the tribe Plucheeae. Analysis of sequences of the plastid *trnL* region and the nuclear rDNA Internal Transcribed Spacers (ITS) indicate that, apart from a few African species belonging in the genus *Laggera* of tribe Plucheeae, *Blumea* constitutes a monophyletic group with three well-supported branches: 1) the single species *B. balsamifera* (L.) DC., a shrub or small tree widespread in tropical Asia, 2) the Densiflora clade, a group of medium-sized perennial herbs and subshrubs, growing in the evergreen forests of tropical Asia and 3) the Lacera clade, a variable group of mostly weedy, often annual species, usually growing in open forests and fields in tropical Asia, Australia and Africa.

P1057. *Frolovia*, *Himalaiella* and *Lipschitzia*: Three small genera in *Cardueae* (Compositae) separated from *Saussurea*

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Recent molecular as well as morphological studies suggest that the large genus *Saussurea*, consisting of approximately 350 species, is a paraphyletic assemblage. So far, three small genera *Frolovia* (5 species in Altai and Tian Shan), *Himalaiella* (11 species from Western to Eastern Himalaya and China) and *Lipschitzia* (2 species in Western Himalaya and Karakorum), have been recognized as distinct from *Saussurea*. In this study, DNA sequences from the nuclear ribosomal ETS, ITS and from the chloroplast *trnL/trnF* region of representative species of these genera, as well as from *Saussurea*, *Jurinea* and *Dolomiaea*, were analysed. The cladograms resulting from ETS and ITS sequence data confirm the paraphyly of the former *Saussurea* s.l. The *trnL/trnF* intergenic spacer shows very little variation in all examined species, however, a remarkable common 22bp insertion in *Dolomiaea*, *Jurinea*, *Frolovia*, *Lipschitzia* and *Himalaiella* suggests a monophyletic origin of these genera. Morphological data, especially from achenes and pappus, were used to characterize the three genera and are found to correspond well to the molecular results.

P1058. Genetic relationships among members of *Centaurea* sections *Acrolophus* and *Phalolepis* (Compositae) as inferred by RAPD markers

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Shape of involucre bracts has traditionally been used as a discriminative character between *Centaurea* sects *Acrolophus* (Ac) and *Phalolepis* (Ph). However, morphological resemblance and adjacent distribution of critical species pairs (one in Ac, the other in Ph) question sectional distinction. We examined 18 members and 22 populations of the two sections by RAPD markers; among them critical groups as *C. attica* (Ac) - *C. subsericans* (Ac) - *C. pseudocadmea* (Ph), *C. cithaeronea* (Ph) - *C. musarum* (Ph), *C. incompleta* (Ac) - *C. lithochorea* (Ph) and *C. niederi* (Ac) - *C. heldreichii* (Ph) - *C. leonidia* (Ph). Members of *C.* sects *Acrocentron*, *Microlophus* and also *Cyanus* and *Carthamus* were included for comparison. A total of 11 primers were used, out of 15 tested. UPGMA clustering failed to distinguish any deep dichotomy between the two sections, although comparison groups separated well. Interspecific genetic distance increased with geographic distance. Small groups of genetically close taxa such as *C. attica* (Ac) - *C. subsericans* (Ac) and *C. incompleta* (Ac) - *C. lithochorea* (Ph) were formed. Our data do not support distinction between *Centaurea* sects *Acrolophus* and *Phalolepis*.

P1059. Molecular systematics of the *Rhaponticum* group (Compositae, Cardueae-Centaureinae): taxonomic, biogeographic and morphological implications.

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The *Rhaponticum* group comprises about 8 genera and approximately 40 species. Besides the delineation of some genera (*Acroptilon*, *Leuzea*, *Rhaponticum*, *Oligochaeta* and *Ochrocephala*), the circumscription of the group is doubtful. The main point of disagreement is the position within the group of *Callicephalus*, *Karvandarina* and other basal genera from the Centaureinae with conflicting borders with the *Rhaponticum* group. Furthermore the phylogenetic relationships within the *Rhaponticum* group and within the genus *Rhaponticum* are largely unknown. To resolve relationships at the base of Centaureinae and within the *Rhaponticum* group, DNA sequences were generated from nuclear regions (ITS and ETS) as well as from cpDNA region (*trnL-trnF*). Our results suggest that *Rhaponticum* in its present circumscription is paraphyletic, which has deep nomenclatural and taxonomic implications. Phylogenetic reconstruction reveals a different delineation of the group, and points out that it is necessary to revise classification within the genus more in accordance with evolutive history.

P1060. Systematics and Biogeography of *Klasea* (Asteraceae: Cardueae-Centaureinae)

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Klasea is a genus comprising ca. 45 species which is characterized by features mostly regarded as plesiomorphic within Centaureinae. It is traditionally included in *Serratula*, but it has been shown that such a treatment would make *Serratula* polyphyletic. Nearly all species of *Klasea* were investigated by means of nrDNA ITS and ETS sequences, morphology, and geographical distribution. Within the genus, three larger monophyletic groups (assigned to sections *Klasea*, *Demetria*, and *Schumeria*) and six additional clades comprising only one or two species each could be detected.

Centers of species richness are found in the mountains of Central Asia, in the Irano-Turanian region (eastern Anatolia to northeastern Iran), and in the Iberian peninsula. Whereas the Central Asian species all belong to sect. *Klasea* and the Iberian species to sect. *Demetria* (plus one species to sect. *Klasea*), representatives of all groups occur in the Irano-Turanian region. Thus it is very likely that the origin of *Klasea* and the first radiation within the genus occurred there.

P1061. Trichomes in the tribe Lactuceae (Asteraceae) - systematic implications

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Although the infratribal classification of the Lactuceae has been the focus of several studies, relationships between the genera are not yet fully resolved. While trichome characters have been shown to be an important tool in taxa delimitation in many plant families, a detailed study on trichomes within the Lactuceae was still lacking. We assess here the contribution of trichomes to the clarification of their relationships. Trichome diversity was investigated on vegetative organs (stems and leaves) of 135 representatives of 53 genera by light and scanning electron microscopy. According to their morphology and anatomy, they were classified into 8 types and several subtypes. Presence or absence of particular trichome types was scored at genus level, phenetic analysis was performed, and the results were compared with other studies. The previously supposed monophyly of exclusively American genera and affinities between several other closely related genera were confirmed. A new circumscription of the subtribe Hieraciinae is proposed, which is in accordance with recent molecular data.

P1062. Molecular evidence for allopolyploid origins of *Carthamus creticus* and *C. turkestanicus* (Asteraceae, Cardueae)

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The allopolyploids *Carthamus creticus* and *C. turkestanicus* are noxious weeds with wide Mediterranean distributions, and *C.*

creticus has also colonized all other Mediterranean climatic regions of the world (Argentina, Australia, California, Chile and South Africa). Previous morphological and cytological studies have suggested that the progenitor lineages of *C. creticus* ($2n = 64$) are represented by *C. lanatus* ($2n = 44$) and *C. leucocaulos* ($2n = 20$), and that those of *C. turkestanicus* ($2n = 64$) are represented by *C. lanatus* and *C. glaucus* ssp. *glaucus* ($2n = 20$), which also agree with their distribution areas. Here we address the origin of these two allopolyploids and their relationships with other species of the *Kentrophyllum* group using cloned fragments of two non-coding regions of the nuclear RNA polymerase genes (RPC2 and RPD2), one non-coding chloroplast DNA region (*trnH-psbA*) and DNA fingerprints (random amplified polymorphic DNAs, RAPDs). The results support the original hypotheses of allopolyploid origins, and both allopolyploids were genetically most similar to the progenitor lineage that contributed the highest number of chromosomes, *C. lanatus*.

P1063. Generic delimitations in Inuleae-Inulinae (Asteraceae)

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The Inuleae subtribe Inulinae contains approximately 300 species in c. 30 genera distributed in the Old World. The phylogeny of the group has been studied on both morphological and molecular data, but many questions regarding the monophyly of the genera have not been adequately addressed, especially with respect to the large genera *Inula* (about 100 spp.) and *Pulicaria* (about 80 spp.). In the present study, a large number of species are included, representing almost all genera of the subtribe and most of the morphological variation within *Inula* and *Pulicaria*. In order to clarify the circumscription of the genera, DNA sequences from two cpDNA regions were analyzed. *Inula* was found to be paraphyletic including also genera such as *Telekia*, *Carpesium*, and *Pentanema*. *Pulicaria* and *Pentanema* were polyphyletic. The small genera *Jasonia* and *Dittrichia* were found to be ingroups in *Pulicaria* and the species of *Pulicaria* section *Francoeuria* belonged to another clade and is better recognized as a separate genus.

P1064. Sporoderm ultrastructure of different pollen types in Asterales

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Pollen grains of some members of the Campanulaceae s.l. (Cyphoideae, Sphenocleioideae, and Campanuloideae) and Asteraceae (Asteroideae and Cichorioideae) were studied with SEM and TEM. The family Campanulaceae s.l. demonstrates pollen of different apertural types, from the polycolpate to polyporate. Each subfamily of the Campanulaceae is characterized by a certain palynotype. In the other family of this order, Asteraceae, only tricolporate pollen occurs. The Campanulaceae and Asteraceae which have tricolporate pollen differ by exine ultrastructure. In the Campanulaceae the spines of the investigated pollen are solid whereas in Asteraceae the spines can have 1 or 2 cavities in their bases. The architecture of the sporoderm ultrastructure on a familial level is well discerned. The subfamilies of both families have insignificant differences in ultrastructure of the pollen even between different apertural types in the Campanulaceae.

P1065. *Leontodon tuberosus* and *Tragopogon floccosus* - new species to the Bulgarian flora

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Currently, targeted research on Lactuceae has been undertaken in Bulgaria. Special attention has been given to poorly studied areas in the country. This has resulted in the discovery of several new species from the group.

So far the genus *Leontodon* comprised six species in Bulgaria - *L. autumnalis*, *L. cichoraceus*, *L. crispus*, *L. hispidus*, *L. rilaensis* and *L. taraxacoides*.

Leontodon tuberosus L. (Asteraceae: Lactuceae) is reported as new to the Bulgarian flora. This Mediterranean species was discovered in grasslands, in the southernmost part of the Black Sea floristic region, near the Turkish border. Although locally

abundant the species is restricted to a relatively small area and is under serious threat by the increasing tourism development along the Black Sea coast.

The poster presents the distinguishing morphological characters from the other Bulgarian *Leontodon* species and the chromosome number and morphology. The national conservation status of the species is discussed.

P1066. Morphometric evaluation of infraspecific variability of *Picris hieracioides* (Compositae) in Europe

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Picris hieracioides is a morphologically very variable and taxonomically complicated species. Usually six subspecies have been accepted in recent European Floras (*P. hieracioides* subsp. *hieracioides*, subsp. *grandiflora*, subsp. *longifolia*, subsp. *riellii*, subsp. *spinulosa*, subsp. *villarsii*), but the taxonomic information is incomplete and often contradictory. Forty-seven population samples of *P. hieracioides* were collected from Central, Western and Mediterranean Europe, and analyzed morphometrically. Two main morphologically distinct groups of populations can be delimited: populations growing at lower altitudes in ruderal habitats, and those from more natural mountain habitats. Within the ruderal group, populations from Central Europe are differentiated from those from Sicily and Spain. The mountain group shows morphological differentiation into five groups, which occupy different European mountain ranges: (1) the Carpathians and E Alps, (2) the W Alps, (3+4) the Pyrenees, and (5) Sierra Nevada. These morphologically defined groups will be proved by cultivation experiments and molecular analyses, and related to the described subspecies.

P1067. Biogeographic patterns and affinities of the Pacific island genus *Oparanthus* (Coreopsideae: Asteraceae)

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Oparanthus is a genus of four species endemic to French Polynesia. Two are endemic to Rapa Iti in the Austral Archipelago and the other species are endemic to the islands of Nuku Hiva and Hiva Oa, respectively, in the Marquesan Archipelago. The genus has been variously circumscribed by earlier authors (e.g., in the genus *Bidens*). Recent phylogenetic studies of the Coreopsideae have shown that *Oparanthus* is not closely related to *Bidens*, but rather is sister to the Polynesian genus *Fitchia*. These studies also suggest that *Oparanthus* and *Fitchia* have close affinities with Caribbean genera. This study is a species-level phylogenetic analysis of *Oparanthus* using quickly evolving gene regions (ITS, ETS, and 5s-NTS) from the nuclear ribosomal genome. The goals of this study are to resolve the migration patterns of *Oparanthus* and *Fitchia* among islands of the southeastern Pacific and to further investigate the biogeographic affinities of this Pacific lineage to other members of the Coreopsideae.

P1068. Morphological, cytotaxonomical and embryological studies on the genus *Taraxacum* Weber (Asteraceae, Lactuceae) in Calabria (S Italy)

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Due to the scarce knowledge of the genus *Taraxacum* Weber in Calabria (S Italy), we carried out morphological, cytotaxonomical and embryological studies on its representatives. Aim of this work, which is still in progress, is to contribute to the knowledge of this difficult genus with basic biosystematic data linked to well defined and localised populations. Our research is based on live plants collected during the year 2004 and on *exsiccata* from the herbarium of the University of Calabria (CLU) and from the Herbarium Centrale Italicum (FI). We have notice of the presence in Calabria of 6 *Taraxacum* sections: *Alpina*, *Erythrosperma*, *Obovata*, *Palustris*, *Scariosa*, *Taraxacum*. Eight populations were studied (coming from Pollino Massif, N Calabria; Catena Costiera and Sila Massif, C Calabria). Morphological data for each population are presented and discussed. The studied populations evidenced several ploidy levels, and often apomixy with *Taraxacum*-type embryo-sac development. It is of particular

interest the presence in Calabria of a population (Sect. *Erythrosperma* Dahlst.) with $2n = 8$ chromosomes, a number never before quoted in literature for the whole genus.

P1069. Phylogenetic relationships among the genera of the subtribe Sonchinae (Asteraceae: Lactuceae) based on ITS and matK sequences

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The subtribe Sonchinae comprises of 13 genera and ca. 130 species and is the most widely distributed subtribe in Lactuceae, with a discontinuous, almost cosmopolitan, phylogeographically very peculiar distribution. Phylogenetic relationships among the genera have been poorly resolved in previous work. In this study, we sampled all representatives of the subtribe and sequenced nuclear ITS of nrDNA and chloroplast matK gene. Both sequences suggest that Reichardia and Launaea diverged first within the subtribe. The genus Sonchus is highly polyphyletic. Three monotypic genera in the Pacific, Embergeria and Kirikianella in New Zealand and Actites in Australia, evolved from some members of Sonchus sections Arvenses and Maritimi. The Juan Fernandez Island endemic Dendroseris, is deeply nested within the subtribe Sonchinae. The woody Sonchus alliance, consists of 6 genera and 31 species, in the Macaronesian Islands is monophyletic, but the closest continental relatives need to be determined. There are several major differences between nuclear ITS and chloroplast matK phylogenies. A phylogeny-based classification of the subtribe Sonchinae will be discussed.

P1070. A taxonomic study on the tribe of Astereae (Asteraceae) in east & northeast of Iran

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Astereae is one of the tribes of Asteraceae. Its phylogenetic situation is between Calenduleae and Anthemideae. In "Flora Iranica" were recorded 14 genus and 33 species from Iran. This investigation is based on morphological study of specimens in east and northeast of Iran (Khorassan Province). *Psychrogeton cabulicus* BOISS. is reported as a new record to the flora of Iran which its distribution in region of Flora Iranica is in Turkmenistan and Afghanistan. 6 species including *Solidago virga-aurea* L., *Erigeron uniflorus* L., *Chamaegeron asterellus* (BORNH.) BOTSCH., *Ch. oligocephalus* SCHRENK, *Conyzanthus squamatus* (SPRING.) TAMAMSCH and *Myriactis wallichii* DC. are recorded as new for the flora of east and northeast of Iran. *Psychrogeton andryaloides* (DC.) NOVOPOKR. ex KRASCH, *Psychrogeton persicus* BOISS. and *Lachnophyllum gossypinum* BUNGE are recognized as rare species. Distribution maps and identification key to the genera and species are provided.

P1071. Molecular phylogenetics of the Edelweiss (Leontopodium, Asteraceae)

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The main distribution of the genus *Leontopodium*, comprising c. 30-40 species, encompasses an extensive and largely contiguous area in Central and East Asia. Two taxa occur, widely disjunct, in the mountains of Central and South Europe (*L. alpinum*, *L. nivale*). The taxonomic status of the European representatives is questionable. Neither ITS nor AFLP data can apparently discriminate between them, although they may well comprise reproductively isolated strains. Sequence data of the ribosomal DNA (ITS and ETS) as well as the plastid *matK* and the *trnL/F* do support monophyly of the genus as well as the species concept. Having many representatives of Asiatic species of *Leontopodium*, as well as morphological, phytochemical and the geographical distribution pattern do support a division of the genus into two sections, *Nobilia* and *Leontopodium*, as suggested by Handel-Mazzetti (1927). Molecular data does not support the monotypic status of the genus *Sinoleontopodium* (Chen, 1985).

P1072. Evolution of non hermaphroditic breeding systems in Asteraceae

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Although most flowering plants are hermaphroditic, other breeding systems (andromonoecy, gynomonoecey, monoecy, androdioecy, gynodioecy, dioecy) have evolved in numerous lineages. Dioecy is considered to have evolved mainly through monoecy or through gynodioecy as intermediate steps. In Asteraceae, hermaphroditism is the ancestral state and andromonoecy, gynomonoecey, monoecy, gynodioecy, and dioecy have evolved secondarily. No formal study has been carried out, however, on the number of independent evolutionary events involved in those changes of breeding system or the pathways followed from hermaphroditism to dioecy. Using a supertree of the family Asteraceae and the comparative method we aim to answer to the following questions: How many times have non-hermaphroditic breeding systems evolved in Asteraceae?, Is gynomonoecey an intermediate step in the evolution of monoecy in this family? Has dioecy evolved via monoecy or via gynodioecy in Asteraceae? Which are the ecological correlates of the evolution of dioecy in this family?

P1073. Botanical and ecological studies of the species of Artemisia L. genus in Azarbaijan-e-Gharbi, Iran

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The largest formation in Irano Turanian zone as well as in Azarbaijan-e-Gharbi is *Artemisia-Astragalus*. Ecological and botanical attributes of habitats, cytogenetic, anatomy, morphology and essential oils were studied and separately discussed after One-way ANOVA in botanical, ecological and systematic aspects for eight populations (six species). Finally, all quantitative attributes were used for multivariable analyzing. Cytogenetically there was two different cytotypes for *A. spicigera* populations; these studies also determined the short arm of the chromosome as a useful trait for categorizing of the studied populations. Most species showed various xerophytic structures that are important in reclamation of similar ecological but disturbed habitats. Resulted cluster showed the dominance of environmental conditions on systematic and taxonomy. Morphological, anatomical and karyotypical traits were used in PCA and cluster analysis to categorize population/species. Three main groups were categorized that it was in long with the last subgenus taxonomy except for *A. incana* population.

P1074. Comparative annual stem anatomy of East-European species of Artemisia L. (Asteraceae): taxonomic value.

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Anatomical features such as the depth of cork initiation, width of rays, presence of secretory canals and phloem fibres are most significant at sectional or subsectional level in genus *Artemisia*. Superficial cork is typically present in sect. *Artemisia* and *Absinthium*. Exceptions are *A. vulgaris* and *A. hololeuca* in which the cork is absent and *A. latifolia* in which the cork is initiated in depth cortex layers. The cork is never initiated in subepidermal layer in sect. *Dracunculus*. Species of sect. *Seriphidium* have both types of cork initiation. Wide rays are present in *A. tshernieviana* and in other semi-shrubby species of sect. *Dracunculus*. Secretory canals are always absent in sect. *Seriphidium* but present in the cortex in members of sections *Artemisia*, *Absinthium* and in some species of sect. *Dracunculus* (excepting *A. vulgaris* L., *A. austriaca*, *A. pontica*) and in secondary phloem in *A. tshernieviana* and related species (sect. *Dracunculus*). These new data support the opinion about origin *Artemisia* from semi-shrubby forms and expand the conception of relationship within sect. *Artemisia* and *Dracunculus* in comparison with molecular data.

P1075. Leaf Epidermal Features In Vernonia amygdalina and Vernonia cinerea

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ABSTRACT

Leaf Epidermal Features In *Vernonia amygdalina* and *Vernonia cinerea*

Vernonia amygdalina *Vernonia cinerea* are tropical plant belonging to the family Compositae and is used widely as vegetable and medicinal plant. The periclinal surface of the epidermis or cuticle of the petiole, mid-rib and of subsidiary cells in *Vernonia amygdalina* is papillose, with papillose, with papillae appearing, in surface view, as striations. That of *Vernonia cinerea* is, however, non-papillose. Four types of trichomes are observable on the two species - regular and irregular T - shaped, uniseriate and sunken, bilobed trichomes. *Vernonia amygdalina* bears irregular T - shaped and bilobed trichomes while *Vernonia cinerea* has regular T - shaped, uniseriate and bilobed trichomes. All the trichomes except uniseriate type are glandular and more abundant on the abaxial surface than the adaxial surface of the leaf in both species. Stomata which are anomocytic and actinocytic also have similar distributional pattern.

P1076. Introduction of the genus *Echinacea* Moench species in Ukraine

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In the O. V. Fomin Botanical Garden of the Taras Shevchenko Kyiv National University has been created the collection of the genus *Echinacea* Moench species: *E. angustifolia* DC., *E. atrorubens* Nutt., *E. pallida* (Nutt) Nutt., *E. purpurea* (L.) Moench, *E. simulata* Mc Gregor, *E. tennesseensis* (Beadle) Small.

First the high introduction ability of the majority of the genus *Echinacea* Moench species has been substantiated. The comparative characteristics of peculiarities of development of investigated species have been carried out. The melliferous productivity of *E. purpurea* in its cultivation on the production plantations under the conditions of Ukraine has been substantiated. First the estimation of microelement contents of cultivated in Ukraine *E. purpurea*, *E. pallida*, *E. angustifolia* has been carried out. In extracts of *E. purpurea* the biologic-active substances of albumen - lectins - have been found. On base of integral estimation of the introduction peculiarities in culture of the investigated genus *Echinacea* Moench species has been established the possibility of their production cultivation in the majority of regions of Ukraine.

P1077. Reproductive biology of two closely related species of *Leontodon* L. (Asteraceae)

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We carried out a reproductive biology study of two closely related *Leontodon* (Asteraceae) species: *L. salzmannii* (Schultz.) Ball (endemic to the Morocco) and *L. muelleri* (Shultz. Bip) Fiori (occurring in Africa, Italy and SE of the Iberian Peninsula). Both species are annual and heterocarpic.

In order to gain insight into their breeding behaviour and to check on the possible sterility barriers, an artificial crossing program was prepared. It showed that self-incompatibility prevails in these species. Moreover cross-compatibility was found in most combinations within populations and between populations belonging to same taxon. Artificial hybridization showed that *L. salzmannii* and *L. muelleri* were cross-compatible. We studied seed-set and germination of seeds obtained after hybridization, as well as fertility, somatic chromosome number and meiosis in hybrid plants.

In the other hand, we observed that *L. salzmannii* and *L. muelleri* differ significantly in germination characteristics.

P1078. Cytogeography of *Pilosella officinarum* (Compositae, Lactuceae) in the Western Carpathians

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Pilosella officinarum is represented by 5 cytotypes (2x, 4x, 5x, 6x, 7x) throughout its distribution area. While the cytotype distribution is well documented for W and NW Europe, only scattered data were published from the rest of the area of this species. In total, 405 plants from the W Carpathians were analyzed by flow cytometry or classical counting within our study. Pentaploid level was found as the most frequent, following by hexaploid one. Mixed populations (5x + 6x) were represented in 8 % of cases. Contrary to the W Europe, tetraploids are very rare in the W Carpathians. The differences in altitudinal distribution were observed; 6x plants

only rarely occur at high elevations in contrast to 5x plants, which are frequent there. Our W Carpathian data agree with the high proportion of 5x and 6x plants found in Scandinavia and in the Alps. Current cytotype distribution patterns probably reflect a postglacial colonization of the tetraploid cytotype from the pre-Alpine region, followed by the rise of ploidy level in the marginal areas in the north of Europe and in larger orophytic systems (Alps, Carpathians, ?Balkan).

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P1079. Chloroplast and low-copy nuclear region DNA evidence on phylogeny of *Arnica* (Asteraceae-Madieae): comparisons with nuclear ribosomal DNA data

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Arnica is a circumpolar genus of 29 spp restricted to the northern hemisphere. Previous taxonomic studies indicate high variability and a reticulate history of hybridization and polyploidy.

Chloroplast rpl16, rps16, trnT-L, and psbA-trnH spacer sequences were found to contain minimal sequence variation relative to variation in nuclear ribosomal transcribed spacers (ITS and ETS) for 26 *Arnica* species. The informative chloroplast DNA characters, however, confirm some of the clades in trees resulting from analysis of ITS and ETS variation. A phylogenetic hypothesis based on combined nuclear and chloroplast DNA data indicates that previous circumscriptions of subgenera in *Arnica* are problematic.

Sequencing of rapidly evolving, low-copy nuclear regions (Nitrate Reductase and RNA polymerase) is being pursued to obtain another line of evolutionary evidence, with the goal of generating a robust phylogeny of *Arnica* and for estimating proposed reticulate evolution of the genus.

P1080. Phylogenetic relationship of the intraspecific variation of *Stevia organoides* (Eupatoriaceae; Asteraceae) based on AFLP.

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Stevia (Eupatoriaceae; Asteraceae) is a large genus containing approximately 230 species, occurring in montane regions of southwestern USA through Central & South America. Mexico is a center of diversity with ca. 100 spp. diverged to annuals, perennials and shrubs. Forty-two species of them include agamosperous polyploids, most of which have wider distribution than sexual diploids. Our previous studies on Mexican *Stevia* using ITS data results in the following. Shrub and perennial-annual clades diverged at the base of phylogenetic tree. Agamosperous polyploids evolved independently in 15 perennial species, but did not evolve in annuals and shrubs. *Stevia organoides* is one of the examples for sympatric speciation with both sexual and agamosperous form and has broad morphological variation in sexual diploids. We focused on the speciation among sexual diploids of *S. organoides*. The various types of ecological and morphological lineages were analyzed based on AFLP analysis. We found that it includes more than 5 biological species. This finding suggests that *S. organoides* is actively radiated to heterogeneous environments of slopes and cliffs in Mexico.

P1081. Origin and relationships of the austral genus *Abrotanella* (Asteraceae) inferred from DNA sequences

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Relationships of the small austral genus *Abrotanella* are uncertain. It was previously included in the Anthemideae or more recently in the Senecioneae. We conducted a phylogenetic analysis of 39 accessions including most species of *Abrotanella* and putative relatives including *Blennosperma*, *Crocidium* and *Ischnea*. These four genera have been considered to form subtribe *Blennospermatinae*. Our results suggest that the *Blennospermatinae* are polyphyletic. *Blennosperma*, *Crocidium* and *Ischnea* form a well-supported clade nested within the Senecioneae, while *Abrotanella* forms another well-supported clade that is distinct from these genera. However, its sister is not

resolved. Molecular dating of *Abrotanella* suggests that the lineage initially diverged during the early Miocene. Secondary species radiations in Australasia and in southern South America that occurred 3 million years ago undoubtedly reflect long-distance dispersal, colonization, and speciation. The divergence estimates are robust to the genes that were sequenced, to changes in the tree topology, and to the method used to estimate divergence times.

P1082. Systematics and Evolution of the Genus *Formania*

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The systematic position of the monotypic shrubby genus *Formania* has been disputed for more than half a century. In this study, its position was assessed by using evidence from morphology and three DNA sequences. Results indicate that the apex of style branch of its disc floret is the "asteroid type", not "styli truncati". This evidence strongly supports the close affinity between *Formania* and the Astereae. Phylogenetic analyses of trnL /F spacer and the trnL intron also indicate that *Formania* has strong affinity with the tribe Astereae. Parsimony analyses of ITS sequences of the Astereae indicate that *Formania* is nested at the basal position of the clade comprising genera of subtribe Asterinae from Eurasia. This clade is only weakly supported. But the placement of *Formania* in the Euraisan Asterinae is justified by some morphological characters. Considering the woody habit and other primitive characters compared with the Eurasian Asterinae, *Formania* seems to represent part of the ancestral stock of subtribe Asterinae that found refuge in the Jinshajiang dry-warm valley after the radiation of the Asterinae in Eurasia.

P1083. Morphological analysis of *Euchiton argentifolius* - *Euchiton traversii* species complex (Gnaphalieae: Asteraceae)

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Euchiton has an Australasian distribution with several species restricted to alpine regions in Australia and New Zealand. There is confusion as to the delimitation of species in the *E. argentifolius* - *E. traversii* complex. This paper resolves taxa by phenetic comparison of populations based on morphological characters, covering the geographic range of the complex. More than 140 specimens of six currently accepted taxa were included in this study. These encompass collections from seven herbaria and material collected in the field. Continuous measurements as well as binary characters and SEM were utilised. A generic split is confirmed within this group and several species are transferred. *Euchiton mackayi* is shown not only to be present in Australia, a significant range extension, previously being thought a New Zealand endemic, but it is also shown to have a strong affiliation with *E. argentifolius*. The outcome of this work is a clarification of the number of members in this alpine group, as well as revised distributions, resulting in a more workable taxonomy that will enable easier identification of these small but plentiful daisies.

P1084. Phylogenetic analysis of *Bulbostylis* Kunth (Cyperaceae) using plastid DNA sequence data.

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Bulbostylis (ca.150 species) occurs primarily in tropical and subtropical regions. It is distinguished from other Cyperaceae genera by the presence of trichomes on the leaf sheath and a persistent style base on the achene apex. The genus belongs to the subfamily Cyperoideae, tribe Abildgaardieae which also includes *Abildgaardia*, *Fimbristylis* and *Nemum*. The relationships among and circumscriptions of these genera in Abildgaardieae have been controversial. *Bulbostylis* have been placed in different taxonomic positions. In order to elucidate species relationships, evaluate existing infrageneric classifications and test the circumscription of *Bulbostylis*, a phylogenetic analysis using cpDNA sequence data (*trnL* intron and *trnL-F* intergenic spacer)

was carried out. 107 species of *Bulbostylis* were included, and *Fimbristylis* (7 species), *Nemum* (2), *Scleria* (2), *Becquerelia* (1) and *Lagenocarpus* (1) were used as outgroup.

P1085. Systematics of the genus *Scleria* Bergius

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Scleria Bergius is a large and important pantropical genus within the Cyperaceae comprising ca. 200 species. It is partly characterised by unisexual flowers in unisexual or bisexual spikelets and the presence of a hard, one-seeded nutlet.

Generic boundaries in this group are unclear, particularly the relationships between *Scleria*, *Acriulus*, *Diplacrum*, *Becquerelia*, *Bisboeckelera* and *Calyptrocarya*. An absolutely essential part of the project therefore is to test the monophyly of the genus *Scleria* and clarify the relationships between *Scleria* and closely related genera.

An understanding of its phylogeny is crucial to the development of a phylogeny for the family as a whole. It will also help in understanding patterns of character evolution in the Cyperaceae and so contribute to an overall picture of evolution in the Monocotyledons.

Preliminary morphological and molecular analysis (*trnL-F* and ITS) have revealed new phylogenetically informative characters and allowed construction of a preliminary phylogenetic tree.

P1086. Systematics of Juncaceae

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Phylogenetic relationships of Juncaceae and related families of Cyperales were investigated using DNA sequence data from the plastid *rps16* intron, *trnL* intron and *trnL-F* intergenic spacer. Results using parsimony analysis of the aligned matrices find Juncaceae and Cyperaceae to be monophyletic families, which form a clade sister to a monophyletic Thurniaceae that includes Prioniaceae, all three clades with 100% bootstrap support. Within Juncaceae the genus *Luzula* is monophyletic with 100% bootstrap support and sister to the rest of Juncaceae. Further groupings within the family indicate that the genus *Juncus* may be monophyletic only with the inclusion of the single-flowered genera of Juncaceae (*Distichia*, *Oxychloe*, *Marsippospermum* and *Rostkovia*; sequences of *Patosia* were not included). Major groupings within *Juncus* are supported by the morphological characters of septate or non-septate leaves and the presence or absence of bracts subtending the flowers, which have been used to define subgenera and sections within *Juncus* in classifications including Kirschner et al. 2002a, b, c. Reference to anatomy will also be made.

P1087. Cyperaceae C₃ and C₄: an overview on the Brazilian species.

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Cyperaceae is one of the few families which both photosynthetic pathways (C₃ and C₄) are present. Cross sections of cataphylls, leaves, bracts, and stems were realized. Fresh and dried samples of 110 species from 24 genera occurring in different ecosystems mainly from NE Brazil were analyzed. Among the studied species, 40 have Kranz anatomy well characterized. Four subtypes were recognized - chlorocyperoid (*Ascolepis*, *Cyperus*, *Kyllinga*, *Lipocarpa*, *Pycreus*, *Remirea*), rynchosporoid (*Rhynchospora*), eleocharoid (*Eleocharis*) and fimbristylid (*Abildgaardia*, *Fimbristylis*, *Bulbostylis*). *Becquerelia*, *Calyptrocarya*, *Carex*, *Diplacrum*, *Fuirena*, *Hypolytrum*, *Lagenocarpus*, *Machaerina*, *Mapania*, *Oxycaryum*, *Pleurostachys*, *Scleria*, *Websteria* are exclusively C₃ genera. *Cyperus*, *Eleocharis*, *Fimbristylis*, *Rhynchospora* are genera with both pathways, however only one species is recognized as a C₃-C₄ taxon (*Rhynchospora globosa*). Species with an uncompleted third sheath and a subtype of Chlorocyperoid named Kranzkette were also recognized among the studied species. No geographical pattern of C₄ species or genera in Brazil is already recognized.

P1088. The genus *Bolboschoenus* in Central Europe: distribution and habitat differentiation

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Formerly broadly conceived species *Bolboschoenus maritimus* (L.) Palla (= *Scirpus maritimus* L.) appeared to be a complex of taxa differing much more in ecology and their distribution areas than in morphological characters. The following species have been found to occur in Central Europe: *Bolboschoenus maritimus* (L.) Palla (saline habitats), *B. planiculmis* (F. Schmidt) T. V. Egorova (wet ditches, temporarily flooded field depressions), *B. yagara* (Ohwi) Y. C. Yang et M. Zhan (fishpond littorals, acid grounds), *B. laticarpus* Marhold, Hroudová, Zákravský & Ducháček (river shores and floodplains, temporarily flooded field depressions), *B. glaucus* (Lam.) S. G. Sm. (river floodplains, warmer regions). Their distribution in Central Europe was mapped based on revision of herbarium collections and authors' field investigations. The *Bolboschoenus* species, which differ in their distribution areas in Eurasia, meet in Central Europe; their areas of distribution within this region correspond to their habitat differentiation.

P1089. Chromosomal evolution of the genus *Eleocharis* (Cyperaceae), based on molecular phylogeny and karyomorphological observations

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The nrITS, nrETS 1f, and *trnL* intron sequence data were used to estimate the phylogeny of *Eleocharis* species, and karyomorphological observations were made on 20 species of this genus. Two major clades were identified in the Japanese *Eleocharis* molecular phylogenetic tree: (1) one including all species of section *Limnochloa*, and (2) the other species of the genus *Eleocharis*. Phylogenetic analysis including both Japanese and North American species also shows strong support for monophyly of the *Limnochloa* clade. The width of the spikelets in species of section *Limnochloa* is the same as that of the culms, and indicates that the relative widths of spikelets and culms are useful characters for classification. Two major clades were supported by karyomorphological data. All species of section *Limnochloa* had very small chromosomes, while sections *Pauciflorae* and *Eleocharis* had large chromosomes. The basic number of sections *Eleocharis* and *Pauciflorae* is thought to be $x = 5$. Chromosomal evolution in the genus *Eleocharis* with diffuse centromeric chromosomes, may be caused by both aneuploidy and polyploidy.

P1090. Taxonomical diversity of Cyperaceae Juss. in Ukraine

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The Cyperaceae is one of the biggest families in Ukrainian flora. The systematic revision and chorological investigation of this family permitted to define taxonomic diversity and distribution within state borders. *Carex demissa*, *C. loliacea*, *C. strigosa*, *C. bunnescens* have been found as new species for Ukraine and proved the presence of *Eleocharis mamillata*. Availability of *Cyperus badius*, *Eriophorum scheuchzeri*, *Schoenoplectus americanus*, *Rhynchospora fusca* have been revised as falsely mentioned for Ukrainian flora. Thus, Cyperaceae family is presented by 19 genus and 149 species. According to the species quantity genus are located in such order: *Carex* - 96 sp., *Eleocharis* - 13, *Schoenoplectus* - 7, *Cyperus* - 6, *Bolboschoenus* - 5, *Eriophorum* - 4, *Scirpus*, *Isolepis*, *Juncellus*, *Cladium*, *Schoenus* in twos, *Scirpoides*, *Blysmus*, *Fimbristylis*, *Pycreus*, *Dichostylis*, *Mariscus*, *Torulinium*, *Rhynchospora* - by 1 only. Intrageneric differentiation of the biggest genus is characterized by following data: subgenus *Carex* - 58 sp., *Vignea* - 26, *Kreczetoviczia* - 7, *Psyllophora* - 5 sp. 35 species of Cyperaceae in Ukrainian flora belong to the rare and threatened species.

P1091. A preliminary taxonomic subdivision of the Cyperaceae family in Venezuela

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The Cyperaceae family in Venezuela occupies the seventh place in species diversity within the angiosperms. As part of the family

revision a preliminary distribution of the species within their taxonomic level is presented, using specialized literature along with the creation of a database which includes the specimens deposited in Venezuelan herbaria (VEN, PORT, MY, MYF, CAR, CORO, MER, MERC, MERF, HERZU, GUY). The family is divided in: 4 subfamilies, 12 tribes, 36 genera, 425 species, 33 subspecies and 20 varieties. They occupy a variety of environments: from dry sea level, savannah, disturbed areas, forests, flooded areas as wetlands, to mountain "páramos" at 4200 m. The Cyperaceae includes 43 endemic species in Venezuela. Examples are: *Carex roraimensis* Steyerl.; *Eleocharis ayacuchensis* S. González & Reznicek; *Eleocharis steyermarkii* S. González & Reznicek; *Everardia vareschii* Maguire; *Koyamaea neblinensis* W.W. Thomas & Davidse; *Lagenocarpus venezuelensis* Davidse; *Mapania aturensis* D.A. Simpson; *Rhynchospora agustiniana* T. Koyama; *Scleria camaratensis* Core, among others.

P1092. Sedges may have edges - but what underlies them? Comparing the anatomy and morphology of sedges

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Sedges, like many other monocots, have limited macro-morphological variation with organs (especially leaves and inflorescences) frequently reduced or cryptic in their external structure. This has led to difficulties in understanding relationships or even in distinguishing related species. The underlying anatomy helps us understand the morphology and evolution of sedges, especially in groups that are scleromorphic. Many Australasian /Gondwanic genera fall into this category. The usefulness of anatomical features has been well understood by cyperologists, as exemplified by the recognition of the correlation of vegetative anatomy with the different photosynthetic pathways found in the family. Examples will be drawn from published and unpublished studies on *Cyperus*, *Lepidosperma*, *Mesomelaena*, *Schoenus*, as well as *Juncus* in family Juncaceae, the putative sister to Cyperaceae, of the continuing value of anatomical characters in interpreting morphology, identifying species and understanding relationships within the sedges and the rushes.

P1093. *Pycreus* P. Beauv. in East Africa; genus or subgenus?

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Taxonomic progress in large genera such as *Cyperus* L. s.l. (600 spp., Cyperaceae) is hampered by an inadequate infrageneric classification with no general agreement on its circumscription. When splitting *Cyperus* s.l. into several subgenera, *Pycreus* P. Beauv. (70 spp.) is one of the larger subgenera, with its highest concentration in Africa. It is treated by some as a separate genus or as part as *Cyperus* s.l. by others. *Pycreus* differs from *Cyperus* by having 2 (not 3) style-branches placed dorsally and ventrally in the flower, and by the lateral compression of the nutlet. Based on these characters *Pycreus* can be considered as a separate genus from *Cyperus* s.s. Discussion rises however whether *Pycreus* is a polyphyletic group, for reduction from 3 to 2 style-branches is common in Cyperaceae. Although molecular studies support the theory that *Pycreus* is indeed a polyphyletic group, it will be considered a valid genus based on its morphological differences, at least for the East Africa region. A synopsis of the East African species will be presented.

P1094. Inferring phylogenetic relationships in *Carex* subgenus *Vignea* (Cyperaceae): impact of nrDNA pseudogenes and paralogs.

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Carex subgenus *Vignea* (Cyperaceae) includes approximately 320 species and is organized into approximately 25 sections. Recent molecular phylogenetic studies have suggested that subgenus *Vignea* forms a well-supported monophyletic group, but relationships within *Vignea* are more problematic. Past classifications of *Vignea* have loosely placed most of the species into sections based on discontinuities in complex morphological characters and recent phylogenetic analyses has suggested that limits of several of these sections do not follow evolutionary patterns. We explore the molecular evolutionary dynamics of nrDNA ITS and ETS to investigate what role ribosomal DNA

paralogs and pseudogenes might play in confounding our assessment of the sectional limits of many androgynous *Vignea*. Additionally, nrDNA inferred polyphyly of several species has prompted investigation of the potential impact of hybridization and lineage sorting among supposedly distantly related taxa.

P1095. A taxonomic study on six sections *Ischnostachya*, *Anomalae*, *Capitellatae*, *Debiles*, *Capillares* and *Molliculae* of *Carex* L. in Korea (Cyperaceae)

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Morphological characters of 14 taxa in 6 sections *Ischnostachya* (one taxon), *Anomalae* (one taxon), *Capitellatae* (five taxa), *Debiles* (one taxon), *Capillares* (one taxon) and *Molliculae* (five taxa) of genus *Carex* (Cyperaceae) were reexamined. The epidermal patterns of perigynium, achene and leaf were investigated by using a scanning electron microscope (SEM) and a light microscope (LM). Morphological characters such as rhizome, spike, width of leaf, leaf sheath, involucre, involucre sheath, beak of perigynium, shape of perigynium shape, and leaf epidermal patterns were useful for the identification of observed fourteen taxa. The number of spike, arrangement of psitillate or staminate spike and presence of bract were very useful characters to divide the treated 14 taxa into two groups, which the first group belonged to sect. *Capitellatae*, the second group belonged to sections *Ischnostachya*, *Anomalae*, *Debiles*, *Capillares* and *Molliculae*. A key based on data was presented here.

P1096. Ecological influences on *Carex* speciation.

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With circa 2000 species widespread in the Northern hemisphere *Carex* contains half of the Cyperaceae species, consequently *Carex* is critical for understanding the evolution of Cyperaceae. In Europe some 200 species of *Carex* occur and these are found in almost every possible habitat. We investigate the habitats of *Carex* in Switzerland where 96 species occur; most of them are found in sunny, wet habitats in mid-altitude (500m-1000m), but there are species present at all altitudes, in wet or dry habitats, in full sun or deep forest shade. These habitat types can be described by physical factors such as pH, water supply, altitude, soil consistency, light availability. In order to establish how phylogenetically conservative these habitats are, we map them over the phylogeny derived from sequence variation in cpDNA (*trnT-L-F* and *matK*). The generalist and specialized species are pointed out and the results are summed up on the poster.

P1097. The complex *Carex muricata* in Europe

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Carex, the fifth big plant genus, has a difficult taxonomy because of the closely related and similar species, among hybridization tends to obscure taxa delimitation. So, in Europe *muricata* group in sect. *Phastoglochin*, includes taxa with overlapped characters. Some works agree in the difficulty of taxa delimitation, and highlight its confuse nomenclature. Our research was based on herbarium material and, field collections mainly from the Alps, Cantabrian and Pyrenean Mountains. Some 1000 sheets were revised; but to perform statistical and numerical analysis, we selected 140 specimens covering the whole geographic range and morphological variation and, ten belonging to the similar *C. otrubae* that easily hybridize with them. We analyzed all relevant characters indicated in former studies, together with other that allow us to distinguish the taxa. The variability of 66 characters was estimated, but only 61 were selected to perform PCA and hierarchical clustering that corroborate five taxa at species level, *C. spicata*, *C. muricata*, *C. pairaei*, *C. divulsa*, and *C. guestphalica* and several subspecies, some of them new.

P1098. Reproductive ecology of *Carex typhina*, a rare sedge in Maine, USA

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Carex typhina is considered rare, threatened, or a species of concern throughout much of its range in eastern North America. In

Maine, the only known location of *C. typhina* is a floodplain forest that has undergone a recent, anthropogenic reduction in spring flooding. We studied 753 individuals at this population to identify potential adverse effects of the change in seasonal hydrology. In 2004 we determined reproductive mode, reproductive output, and germination requirements in different habitats. Although this species was capable of self-fertilization and out-crossing, it was primarily self-fertilized. Large numbers of viable seeds were produced (375 ± 343 , $n = 61$). Average achene weight and percent of perigynia with ripe achenes did not differ significantly between habitats. In an *ex situ* study, seeds germinated readily in shallow water and on moist soil. Light was required for germination, but germination was reduced when light was filtered through leaves. The Maine population is currently vigorous, but the new flooding regime might increase competition from woody plants and thus inhibit *C. typhina* germination.

P1099. The sex of *Carex secalina* (Cyperaceae): from structure spikes to evolution

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In this study we have indicate the usefulness of the traits of life history in recognition of the species evolution. *Carex secalina* is one of three species representing the section *Secalinae* in the subgenus *Carex*. Over the whole range it is considered a rare species, growing in isolated localities in Europe and Asia. In the life history of a sedge *Carex secalina* we have found out that a sex of spikes ranges from mono-zone unisexual through two-zone bisexual - androgynous and gynaeandrous - to bisexual three and four - zone. Bisexual spikes have not been reported for this species and section. In all populations, individuals had generative shoots with bisexual spikes; yet most of the shoots with bisexual three-zone were recorded in a population within man-made habitats. We suggest that it is not the two-zone spikes but multi-zone spikes that are starting point for all spikes forms observed today; evolution to other forms would proceed through simple reduction of particular zones of a complex onflorescence. In the discussion an attempt was made to modify the present model of sex evolution of spikes in genera from the subgenus *Carex* on the basis of ecological studies.

P1100. Studies in some European *Carex*. Taxonomic problems in typifying Hudson's *Carex* names

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Carex is one of the biggest and taxonomically complex genus in the temperate and cold floras of the Northern hemisphere, increasing from 500 to 2.000 species in a century. Its difficult taxonomy, the morphologically similar species groups, and the common hybrids enlarge the problems. In addition, nomenclature is also confuse. In working with sect. *Phastoglochin* we found that Hudson's names have been applied in different senses. So, the aim of our work is to study this genus in his Flora and typify all new names to establish their correct application. A search for original material was made in the possible herbaria BM-SL (located for *C. divisa*), K, LINN, and, OXF and cited books (i.e. we select a Morison's plate for *C. recurva*). For names we were unable to locate original material we select a neotype. (i.e. *C. patula*). Furthermore, we typified other names (i.e. *C. canecens*) because Hudson's concept is not equivalent to the Linnaeus's one. Finally, taking into account the different concept of *C. inflata* in the second edition, we selected a different type for each case. In other cases we only made taxonomic considerations allowing to interpret Hudson's concept

P1101. Phylogenetic analysis of *Carex pisiformis* complex and allied species (Cyperaceae) based on nuclear DNA sequences

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Species of *Carex pisiformis* complex (sect. *Praecoces*) are distributed in eastern Asia, and this complex is one of the most taxonomically complicated groups of sedges in Japan. Different taxonomic systems were proposed. In this study, phylogenetic relationships of *C. pisiformis* complex and allied species were estimated from the internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions of nuclear ribosomal DNA. Two

major clades were identified by molecular data: (1) subsect. Pisiformes, and (2) subsect. Foliosissimae. *C. pisiformis* complex is belong to subsect. Pisiformes, which is considered closely related to subsect. Foliosissimae (Akiyama 1955). These two clades were supported by karyomorphological data. The species of former clades had $2n=56-80$ and small chromosomes, while latter clades had $2n=30-44$ and large chromosomes. Species of subsect. Pisiformes differed from subsect. Foliosissimae by long leaf blades and conspicuous discoid appendage at the apex of achenes. Based on the ITS and ETS data, phylogenetic relationships among species of *C. pisiformis* complex are also discussed.

P1102. Preliminary results concerning Ebenaceae phylogeny

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Ebenaceae s.s. (without the subfamily Lissocarpoideae) is a family of trees and shrubs with characteristic unisexual flowers, a persistent calyx and tasty berries. Some representatives, such as *Diospyros ebenum*, *Diospyros celebica* and *Diospyros melanoxydon*, produce the well-known ebony, used for furniture, musical instruments, etc... (Wallnöfer 2001). Ebenaceae s.s. comprises two genera: the pantropical *Diospyros* and the smaller genus *Euclea*, limited in distribution to Africa, Arabia and Australia. The molecular study at hand is based on a sampling of 55 species using one nuclear (ITS region), the *rps16* intron and three cpDNA spacers (*trnL-trnF*, *trnH-psbA* and *atpB-rbcL*). Preliminary results confirm the paraphyletic nature of *Diospyros* at generic level as two *Diospyros* sections (*Forsteria* and *Royena*) cluster together with representatives of genus *Euclea* in Parsimony and Bayesian analyses. Also, *Royena* seems to be paraphyletic: it forms a clade with section *Forsteria*.

P1103. Differentiation of seeds in the genus Erica

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Erica belongs to the most numerous species of the Ericaceae family. It counts almost 800 species. Most of them occur on the relatively small area in southern Africa. Despite a great interest of researchers in this species, many problems still remain unsolved. One of them is a limited knowledge of the structure of seeds. The presented thesis contains the results of the preliminary stage of research of the seeds of this genus. It contains the description of the morphological and anatomical structure of seeds of 33 species from the *Erica* genus. The taxa chosen for the research originate from the Republic of South Africa, from the floristic center of genus diversity. The structure of seeds was prepared on the basis of observations using the light microscope and the scanning electron microscope. The analysis conducted on the basis of the isolated features shows clearly separate morphological groups, varying in the sculpture and thickness of the seed skin. The morphological diversity of this group of species does not agree with the currently adopted taxonomic division within the genus.

P1104. Phylogenetics of Balsaminaceae based on *atpB-rbcL* spacer sequences

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Balsaminaceae are a morphologically diverse family consisting of 1000 representatives that are mainly distributed in the Old World tropics and subtropics. In order to understand the evolution of this family, we obtained chloroplast *atpB-rbcL* sequences for 87 Balsaminaceae species and 5 outgroup species. Phylogenetic reconstructions using parsimony and Bayesian approaches provided a well-resolved phylogeny in which the sistergroup relationship between *Impatiens* and *Hydrocera* is confirmed. The overall topology of *Impatiens* is strongly supported and most of the delimited clades can easily be associated with a specific area of distribution. Based on our results, we assume that *Impatiens* has originated in South China from where it initially colonised the adjacent regions and afterwards dispersed into North America, Africa, India, Southeast Asia and the Himalaya. Despite the good resolution, it remains difficult to find morphological synapomorphies for the different clades that we find in the *atpB-rbcL* tree.

P1105. Molecular phylogenetics and patterns of seed evolution in *Lysimachia* (Myrsinaceae)

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The genus *Lysimachia* (ca. 150 species) was recently transferred to the family Myrsinaceae based on molecular phylogenetic data. Phylogenetic analyses of ITS, *trnL-F*, and *ndhF* sequences have shown that *Lysimachia*, as understood in traditional classification systems, is likely to be paraphyletic. We have investigated seed morphological characters of 40 *Lysimachia* species from across the genus as well as of representatives of other genera that have been resolved close to or within *Lysimachia* in previous molecular studies. We used a molecular phylogenetic framework based on published (i.e., ITS and *trnL-F*) and unpublished (i.e., *ndhF*) sequence data for a comparison of seed morphology and phylogeny. Our main goal was to find out whether patterns of seed evolution are congruent with the recently found phylogenetic relationships. We also discuss the significance of single morphological character complexes to reveal additional phylogenetic relationships within the genus *Lysimachia* and related taxa.

P1106. Morphological and molecular support for the classification of *Gilia* (Polemoniaceae).

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Gilia Ruiz & Pav. (Polemoniaceae) has been and remains taxonomically controversial. Comparative DNA sequence data conflict with classifications of the family (and with circumscriptions of *Gilia*). Some authors suggest that *Gilia* is polyphyletic. V. E. Grant has suggested that non-molecular data available are sufficient to erect a phenetic classification in Polemoniaceae and that based upon this evidence *Gilia* is monophyletic. Here I examine this morphological evidence, using phenetic (UPGMA, Neighbor joining) and parsimony. Molecular phylogenetic analyses of nrITS and *cptrnL-F* region DNA sequences are presented, with broader sampling of *Gilia*. Groups based on phenetic methods differ significantly from Grant's and the Porter and Johnson classifications, but the two later classifications fit the non-molecular data equally well. The Porter and Johnson classification is also supported by molecular data. Phenetic and phylogenetic analyses provide evidence that *Gilia* s.l. is not monophyletic.

P1107. Diversification of *Erica* L. (Ericaceae) in South Africa's Cape Province

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Extensive analyses of morphological characters of *Erica* L., the largest genus in Ericaceae and in the flora of Southern Africa, have been undertaken to date. The center of diversity of *Erica* L. lies in the southern Cape Province; only 76 of the 860 recognized species are found in the remainder of Africa, Europe, and the Middle East. Previous molecular studies indicate that the South African species are derived. In this study molecular analyses of data from the nuclear genome (*leafy* and *rpb2*) are employed to investigate monophyly and resolution of intrageneric relationships of this taxon. Parsimony analyses of sequence data from 69 species of *Erica* from the southern Cape differing in pollination syndrome, flower morphology, geographical distribution, and soil type preferences are carried out. Indehiscent and dehiscent fruit type is taken into account as this has been a factor for inclusion of the previous "minor" genera into *Erica* L. Species sampling for this study was done to represent the most recent classification including 41 sections. Results of our analyses indicate that indehiscence as well as bird pollination has likely arisen multiple times within the group.

P1108. Palynological variation of Marcgraviaceae

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A survey of the palynological variation of Marcgraviaceae is given based on LM, SEM and TEM observations from all seven currently accepted genera. Features of taxonomic importance within the family are pollen shape, sexine sculpturing, and ultrastructure of

the pollen wall. The genus *Marcgravia* can be distinguished from all other genera by the presence of small grains (15-25 µm) with a high percentage of reticulate sexine patterns and a thin nexine, supporting the division of the family into two subfamilies. Furthermore, *Sarcopera* could be defined by the presence of an oblate spheroidal to even suboblate shape, while *Ruyschia* and *Souroubea* typically show prolate spheroidal to subprolate pollen grains. Another important character is the presence of a thick foot layer, which is characteristic of the genera *Norantea*, *Sarcopera* and *Schwartzia*. From a palynological point of view, Marcgraviaceae have many features in common with the related Tetrameristaceae/Pellicieraceae, but they clearly differ from Balsaminaceae, which are also close to Marcgraviaceae based on DNA sequence data.

P1109. Some aspects of reproductive biology of *Rhododendron schlippenbachii*

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Rhododendron schlippenbachii is a valuable ornamental shrub, which produces a lot of viable seeds under its natural conditions. Under introduction conditions in Saint-Petersburg its flowering and real seed productivity are poor. Embryology and reproductive biology of *R. schlippenbachii* are studied weakly. Data available on other species of this genus allow to suppose that one of the causes of low seed productivity of the plants in St-Petersburg is winter and spring frosts. However the disturbances in microspore development that occur right after the meiosis completion (long before the period of steady frosts) indicate the effect of other factors, in particular, the changes of air and soil humidity. Relatively high quality of mature pollen (fertility is up to 68%, viability - 31-55%) while there is a high amount of abnormal microspores allow to suppose that either some microspores degenerate during microsporogenesis or flower buds with high amount of abnormalities stop their development.

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P1110. Special traits in the inflorescences of Marcgraviaceae - taxonomy and pollination patterns in the neotropical family

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A characteristic synapomorphy of the neotropical Marcgraviaceae are extrafloral nectary cups of various shapes and arrangements within the inflorescence. The evolution of these unique structures implies close interactions with the respective pollinators. Unfortunately knowledge on pollination ecology is rather scarce: very few taxa are intensively studied in their natural habitats. However, inflorescence geometry, colour patterns, and time of anthesis give hints towards the predominant pollinators that may include insects, birds, bats and arboreal mammals. In contrast, there are observations of autogamy, yet cleistogamy in *Marcgravia*. Therefore, generalisations are not expedient and thorough field studies are necessary to reveal the pollination systems which may differ within genera and are instrumental in understanding the evolution of this group.

Based on monographic studies the delimitation of the seven genera and selected characters are discussed in light of the different pollination syndromes exemplified by this family.

P1111. Peculiarities of mucilage-producing cells distribution in some Ericaceae

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Mucilage producing cells (MPC) are wide spread in plants. A general tendency can be traced in MPC distribution within tissues and organs of higher plants. In primitive Dycots MPC occur in leaf epidermis and in developed ones in seed epidermis. The difference in MPC distribution can be traced within one family. In Ericaceae, in Rhododendroideae and Ericoideae MPC were found in leaf epidermis, in Vaccinioideae in exotesta. The species, investigated with TEM *Loiseleuria*, *Erica*, *Calluna*, *Arbutus*, *Vaccinium*, *Oxycoccus* differed in mucilage structure. In investigated Ericaceae a relation is found between MPC location and presence of protein containing plastids (PCP). Such relation we also found in other families. In Ericaceae species with MPC in leaf epidermis PCP were found in parenchyma cells of xylem

group in petiole. In species with MPC in exotesta PCP were found in parenchyma cells of phloem group in fruit stalk. The investigated species did not in protein inclusion ultrastructure in plastids. Investigation granted by RSCI (project N 04-04-49417).

P1112. Phylogenetic relationships among Sapotaceae (Ericales) in Australia and Oceania: molecular evidence for generic polyphyly and repeated dispersal

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The phylogeny of a representative group of genera and species from the Sapotaceae tribe Chrysophylleae, mainly from Australia and New Caledonia, was studied by jackknife analyses of sequences of nuclear ribosomal DNA. The phylogeny conflicts with current opinions on generic delimitation in Sapotaceae. *Pouteria* and *Niemeyera*, as presently circumscribed, are both shown to be nonmonophyletic. Earlier classifications in which more genera are recognized may better reflect relationships among Australian and New Caledonian taxa. Hence, there is need for a revision of generic boundaries in Chrysophylleae. Section *Oligothea* have been recognized as the separate genus *Planchonella*, a monophyletic group that needs to be resurrected. Four clades with moderate to strong support in our jackknife analysis have a group of Australian species that is sister/basal to a relatively large monophyletic group of New Caledonian endemics, suggesting multiple dispersal events between this small and isolated tropical island and Australia. We also found an interesting case of a relatively recent and rapid radiation of several lineages of Sapotaceae within New Caledonia.

P1113. Evolutionary aspects of morphological characters of some European species of *Erica* L. (Ericaceae)

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A set of selected morphological characters with evolutionary information is used for the establishment of a phylogenetic approach to the species of five sections of European species of genus *Erica* that are treated as a monophyletic group including the following species: *E. ciliaris*, *E. cinerea*, *E. maderensis*, *E. terminalis* and the *E. tetralix* group (*E. andevalensis*, *E. mackaiana* and *E. tetralix*). Character selection and establishment of primitive state were determined using the following criteria: 1. Character evolution in the family extracted from different studies. 2. Evolutionary suggestions mainly on bract and bracteoles position given by E.G.H. Oliver in African species of *Erica*. 3. The principles of vestigial functional organs as the functionality of bracts or sepals in primitive species. Cladogram resulting from this study shows *E. ciliaris* as a primitive species of the group and *E. cinerea* as the most evolutionated species. From these results, evolutionary considerations are made in terms of ecological requirements and geographic distribution. Infrageneric systematics are discussed.

P1114. Pollen morphology of *Enkianthus* Lour. (Ericaceae) and its taxonomic significance

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Pollen morphology of 13 taxa and 10 species from about 12 species of *Enkianthus* were examined by light and scanning electron microscopy. Anderberg's infrageneric classification of *Enkianthus* (1994) was reexamined. The pollen grains are monad, oblate spheroidal to prolate or circular in shape, and 3- to 5-colpor(oid)ate. Exine sculpture varies from granulate to coarsely rugulate-psilate. Intraspecific geographical difference in palynological characters is found in *E. deflexus*. The sections are clearly differentiated into two distinct groups, *Enkiantella* - *Meisteria* and *Andromedina* - *Enkianthus*; however, it seems to be difficult to differentiate within these sections on the basis of palynological characters only. The members of *Enkiantella* - *Meisteria* group with 3-colporate pollen grains are the primitive species in this genus. The major evolutionary trend in exine sculpture from finely verrucate-rugulate to coarsely rugulate-psilate, is suggested within *Enkianthus*. Anderberg's infrageneric classification comparatively best suits the present result with some exceptions.

P1115. The analysis of transition between two *Primula vulgaris* subspecies

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The common primrose (*Primula vulgaris*, Primulaceae) consists of several subspecies, two of them (subsp. *vulgaris* and subsp. *sibthorpii*) form a transitional zone in the Russian Black Sea coast, near Tuapse and Sochi. These two subspecies are growing together and even sometimes produce mixed populations, but generally subsp. *sibthorpii* (with violet or pink flowers) grows in lower altitudes than subsp. *vulgaris* (with yellow or white flowers). To test the hypotheses about geographical and spatial distribution, the morphometric material (more than 2310 plants were measured) is collected. We found that there is significant trend to increase the proportion of violet and pink-flowered plants in the populations from north-east to south-west. It is possible to divide populations on "north" and "south" forms. The length of transitional zone is about 30 km along coastal line. However, there is no significant morphological differences between colour forms. There is also the weak correlation between flower color and altitude.

P1116. Marcgraviaceae: resolution of interspecific and intergeneric differences in unresolved molecular polytomies using flavonoid distributions

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Marcgraviaceae is a distinctive neotropical family in the Ericales with approximately 130 species distributed from the Caribbean and southern Mexico to northern Bolivia. Previous *ndhF*, *trnL-F*, and *rbcl* sequence comparisons resolved two major lineages within Marcgraviaceae corresponding to subfamilies Marcgraviaceae and Noranteoideae. The monogeneric Marcgraviaceae is united by both morphological synapomorphies and molecular data. In contrast, subfamily Noranteoideae appears to be delimited by plesiomorphic morphological characters but is supported as monophyletic by molecular data. Despite considerable morphological differences among taxa of Noranteoideae, cpDNA sequence data do not resolve some currently recognized genera as monophyletic groups. This study examines flavonoid distributions in an attempt to resolve molecular polytomies in the family. Preliminary data reveal flavonoid patterns that provide some resolution of intergeneric and interspecific relationships.

P1117. Establishing *Eschscholzia californica* as a model plant for basal eudicots

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Studies in model plants show considerable variation of developmental mechanisms that are attributable to evolutionary changes. In our attempts to elucidate development and evolutionary patterns of dissected leaves, we are establishing *Eschscholzia californica* as a model organism for basal eudicots, in order to help bridging the gap between well investigated monocots such as *Zea* and *Oryza*, and core eudicot model plants. Our previous *in situ* expression studies in *Eschscholzia* indicated that leaf dissection may be under redundant control of *FLORICAULA* and *KNOX* genes in basal eudicots, and that redundancy may have been reduced in specific core eudicot lineages. To further explore *Eschscholzia* leaf development, we attempt to modulate gene expression through *Agrobacterium*-mediated transformation and through Virus-induced gene silencing (VIGS). Furthermore, we are investigating the role of hormones in leaf development of *Eschscholzia* since auxins and gibberellins are known to interact with both *FLORICAULA* and *KNOX* genes in core eudicots.

P1118. Phylogenetic Study of Menispermaceae

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The family Menispermaceae, a major group of basal Eudicot, is mostly represented by tropical climbers belonging to about 500 species. Its phylogeny is very badly understood since there is no recent revision of the group. We therefore provide here the first results of a morphological study tending to fill this lack. Characters of leaves, inflorescences, flowers, fruits and pollen were used. A special attention was given to the endocarp, that are traditionally

used as a discriminating character of the different tribes. We described the endocarp structure through morphometrics and questioned homologies such as the condyle (a cavity of endocarp). We also begun a large-scale study of Menispermaceae wood anatomy on specimens from all around the world. The large fossil record of this family, quite rich in the Early Tertiary of northern hemisphere temperate regions, brings palaeobotanical information in this study. A parsimony analysis allows us to process the whole dataset. It appears that some reassessments have to be made; The new phylogeny permits study of character evolution and biogeographical considerations.

P1119. Patterns and Rates of Nucleotide Substitution in Angiosperms

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We have reliably aligned reciprocally closest homolog pairs for two eudicots (*Gossypium*, *Arabidopsis*) and two members of the Poaceae (*Oryza*, *Zea*). Using reciprocal outgroups to polarize molecular evolutionary events, we demonstrate that patterns of nucleotide substitution differ between eudicots and the Poaceae at fourfold degenerate codon sites (FDCS's). While base composition of FDCS's in *Gossypium* and *Arabidopsis* closely parallels that of introns from the same genes, *Zea* and *Oryza* exhibit FDCS composition distinct from that of introns, suggesting operation of a cryptic mode of selection. Our analysis provides the first robust estimates of neutral rates of transition and transversion substitutions in angiosperms, as well as variance in rates among a large set of genes. These data permit us to address the divergence of the Poaceae from eudicots as well as other divergence events.

P1120. Monophyletic origin and Adaptive Radiation in Hawaiian Chamaesyce (Euphorbiaceae) based on ITS and 5S-NTS sequence variation

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Chamaesyce is a cosmopolitan genus of ca. 250 species. The Hawaiian complex constitutes 16 endemic species that have radiated from a herbaceous weedy colonist into a spectacular assemblage of small shrubs to trees. Habitats include coastal strand, dry shrubland and forests, and rainforests up to 2000 m elevation. All species manifest the C₄ photosynthetic pathway, and are the only such plants to occur in tree form or in wet, understory habitats. Phylogenetic analysis indicates species from Kauai (the oldest island at 5.5 my) are basal and that taxa have followed the stepping stone model of dispersal across the younger islands. Clades include species from all islands suggesting back dispersal or later speciation events. The eight varieties of *C. celastroides*, the most variable species, is paraphyletic and in need of taxonomic revision. Intrapopulation variation of some species has been also been examined and with variation that exceeds that found in any other Hawaiian radiation.

P1121. Pollen morphological trends in Euphorbiaceae

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Pollen materials obtained from fresh and herbarium specimens of 68 genera and 216 euphorbiaceae species found in Thailand were acetolysed. Measurement and morphological observations were made under a Nikon AFX 35, using a x10 eyepieces and x100 immersion objective. All measurement was based on at least 10 pollen grains. External ultrastructures of pollen grains were observed by scanning electron microscope. The SEM micrographs were taken using a JEOL JSM 5410 LV. The permanent slides of the pollens were kept in Kasin Suvatabhandhu Herbarium, Department of Botany, Chulalongkorn University. A conspicuous morphological diversity of euphorbiaceae pollen was evident from 61 pollen types constructed in the present study. They were mainly based on the various types of apertural system and ornamentation. Based on these diverse pollen types, nineteen morphological trends were proposed.

P1122. Phylogenetic Relationships within the Subtribe Euphorbiinae (Euphorbiaceae) Based on Nuclear Ribosomal ITS and Chloroplast *ndhF* Sequence Data

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Phylogenetic relationships within Euphorbiinae were inferred from analyses of the nuclear ribosomal ITS and the chloroplast gene *ndhF*. Relationships inferred from two data sets are mostly congruent and support for the monophyly of subg. *Esula*, *Chamaesyce*, *Euphorbia* and *Lacanthis*, while subg. *Agaloma* and subg. *Eremophyton* are polyphyletic. African succulent Euphorbiinae are mostly divided into two independent groups: spiny group I, forming a strongly supported clade with three subclades (subg. *Euphorbia*, subg. *Lacanthis* and *Monadenium*+*Synadenium*); and non-spiny group II, containing sects. *Meleuphorbia*, *Medusae*, *Treisia* and *Pseudacalypha*. In the ITS tree, the non-spiny clade is sister to the rest of the Euphorbiinae, whereas the basal position is displaced by subg. *Esula* in *ndhF* trees. The South American *Agaloma* sect. *Stachydium* was not nested within the major *Agaloma* clade, but it formed a sister group to *Pedilanthus*. In both analyses, subg. *Poinsettia* is nested within the core *Agaloma* clade, while subg. *Chamaesyce* forms a sister group to core *Agaloma* group. Eurasian subg. *Esula* forms two subclades, corresponding to sect. *Esula* and sect. *Tithymalus*.

P1123. Taxonomic revision of *Croton* sect. *Lamprocroton* (Müll. Arg.) Pax. (Euphorbiaceae)

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Croton (Euphorbiaceae) is a pantropical genus with more than 800 species in 40 sections. *Croton* sect. *Lamprocroton* is a section whose the boundaries between species are controversial. The main goal of this work is to achieve a reevaluation of the circumscription of the group, through a phylogenetic analysis and taxonomic study of its species, presenting identification key, descriptions, illustrations, comments and geographical distribution. Field work and analysis of collections from the main herbaria housing neotropical specimens have been carried out. Morphologically, the members of *Lamprocroton* are characterized as monoecious or dioecious shrubs, with a lepidote indumentum, entire leaves with no glands, terminal inflorescences that lack bisexual cymules, staminate flowers with 10-15 stamens and pistillate flowers without petals or with reduced petals. 25 species, 3 of them new, are here recognized in the group. Most of the section is South-American, with 2 species in Mexico. The phylogenetic analysis, based on morphological and molecular data, support the monophyly of the section, and allow some hypothesis on evolution of characters in the group.

P1124. Evolution of the *Andrachne* clade (Phyllanthaceae or Euphorbiaceae s.l.)

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The *Andrachne* clade is a novel association comprising eight genera previously classified in five tribes and subtribes. *Actephila*, *Andrachne*, *Leptopus*, *Meineckia*, *Poranthera*, *Oreoporanthera*, *Zimmermannia* and *Zimmermanniopsis* contain 129 species of diverse life forms adapted to habitats ranging from tropical rainforest to desert margins and ericoid alpine meadows. Distributions of three genera are geographically disjunct between the Old and the New World. The preliminary results of our phylogenetic study using ITS and *matK* sequence data of 19 taxa are largely congruent. *Zimmermannia* and *Zimmermanniopsis* are embedded in the *Meineckia* clade. *Andrachne* and *Leptopus* are separate lineages, but *Andrachne cuneifolia* and *Andrachne ovalis* are distinct from both. Dating the nodes of the phylogenetic tree and performing a biogeographical analysis will contribute to a greater understanding of the evolution of these taxa in space and time, particularly their adaptation to specialised ecological niches.

P1125. Multiple colonization events for the Madagascan species of the genus *Euphorbia* L. (Euphorbiaceae)

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The subcosmopolitan genus *Euphorbia* L. is one of the largest plant genera on earth. Despite numerous studies, its taxonomy is still obscure, it certainly contains at least 2000 species but a lot of novelties are waiting to be described. The Madagascan flora alone comprises nearly 8% of the world's *Euphorbia* species, with ca. 170 known species and varieties, almost all of them restricted to the island. This work aims to define the main Malagasy species groups, placed in a global context to clarify the taxonomy of the group while defining affinities of the Madagascan flora. This is a first step towards a better understanding of the infrageneric classification. This poster is based on a paper in prep (Thomas Haevermans, Victor Steinmann, Vincent Savolainen, Petra Hoffmann, Emile Randrianjohany and Jean-Noël Labat). In this paper, both maximum parsimony and Bayesian analyses of ITS regions are compared, and the evolution of some morphological characters is discussed.

P1126. Molecular Phylogeny of *Euphorbia* subgenus *Esula* (Euphorbiaceae) based on nrDNA ITS region and plastid DNA *matK* gene sequences

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NrDNA ITS region and plastid DNA *matK* gene were sequenced to assess the monophyly and phylogenetic relationships of twenty eight taxa of *Euphorbia* subgenus *Esula* species. Three species of *Euphorbia* subgenus *Chamaesyce* were applied as the outgroups. NrDNA ITS region (ca. 0.6-0.7kb) were sequenced with two primers (ITS5 and ITS4). And plastid *matK* gene (ca. 1.7-1.9kb) were fully sequenced with previously reported seven primers (from M. Lavin), and another newly designed five internal primers to adjust the clonal sequences. Phylogenetic analyses were carried out with 220 phylogenetic informative sites. Also, MP tree (CI=0.812, RI=0.883) was produced and 1,000 replicate Bootstrap analysis were performed to confirm the tree strength on each node. Based on the nrDNA ITS region and plastid *matK* gene sequences results, Sect. *Helioscopiae* was the sistergroup of subsect. *Galarrhoei* and subsect. *Verticillatae*. And the taxa of subsect. *Esula* represent a monophyletic group. Even though the heterogeneous clades combination was found among sect. *Tithymalus*, This result demonstrate the monophyly of subgenus *Esula* and strongly support previous RAPDs data.

P1127. Systematics and Phylogeny of North-Asian spurges (Euphorbia L.)

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Investigation of spurges (*Euphorbia* L.) from Northern Asia using evolutionary morphology, ecological geography and phylogenetic analysis is completed. The comparative analysis of the various approaches to construction of general system of genus *Euphorbia* is carried out. Conceptions of subgenus, section and subsection in this genus are elaborated, sets of the most important diagnostic features are described for each taxonomic level. A new variant of division of subgenus *Esula* on sections and subsections is proposed. Method SYNAP was improved: new procedure of polymorphic characters is developed, reversion scale was increased. Modeling phylogenetic relations between 29 species of genus *Euphorbia* from section *Esula*, 14 species from section *Tulocarpa* and six species of section *Holophyllum* were executed by method SYNAP using different sets of elementary evolutionary vectors. Than phylogenetic relationship between sections and subgenera was performed. The received phylogenetic scheme was compared with ecological and geographical features of species. It was useful for description of series as a low-level species groups. Works is supported by RFBR.

P1128. Histological studies on cyathia of some *Euphorbia* species

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Euphorbia species have a special inflorescence namely cyathia: it contains a central female flower surrounded by 5 male flowers, 4-5 nectaries and bractea. We studied the histology of the cyathia, with special emphasis on the nectaries in some *Euphorbia* species.

Nectaries have cuticle, epiderm, glandular tissue and parenchyma in all species. The xylem vessels have spiral secondary cell wall thickenings. The nectary cuticle of *E. amygdaloides* and *E. palustris* is thin, that of *E. cyparissias*, *E. esula*, *E. myrsinites* and *E. virgata* living at xerotherm area is thicker. Nectary of *E. palustris* has the highest epidermal cells and the largest number of cell rows of glandular tissue (6-7) which varied from 1 to 4 in the other plants. Cells of glandular tissue are isodiametric in all species except of *E. cyparissias*. Biggest cells of glandular tissue were detected in the nectary of *E. virgata*. The histological structure of the nectary was specific for the studied plant species.

P1129. Taxonomic studies of the two Nigerian varieties of *Ricinus communis*

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Ricinus L. (Euphorbiaceae) is a monotypic genus represented by *R. communis* in Nigeria. It comprises two distinct varieties which may grow either in gregarious or solitary formations. The distinguishing features of the species are colour of vein, petiole and stem which may be green or brown, number of fruit prickles as well as shape of midrib and types of stomata, pollen grains and crystals which may occur either as raphides or localized crystals of calcium oxalate in the epidermal cell lumen. Epidermal wall pattern is usually curved or undulate while cell shape varies from polygonal to irregular. Stomata number per millimetre square ranges from 5-8 on both surfaces and stomata types are anisocytic, anomocytic and paracytic. In the two varieties, polymerase chain reaction (PCR) amplications with random amplified polymorphic DNA (RAPD) primers indicated similarities and differences at 0.59 and 0.41 coefficient levels respectively. *Ricinus communis* has both commercial and medicinal uses.

P1130. Distribution patterns of *Croton* (Euphorbiaceae) in Brazil

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Croton L. is the second largest genus of Euphorbiaceae, with over 1200 species, mostly found in tropical regions. Its main centres of diversity are in the Neotropics, with c. 300 species recorded for Brazil. To identify distribution patterns of the genus in Brazil, we analysed c. 2130 herbaria collections from the total range of 33 species that occur in the State of São Paulo. Natural populations of many species were also visited in order to better understand their lifeforms, habitats and morphological variability. The few endemic species found are trees from the southeastern Atlantic rainforest, one of them restrict to a small area in São Paulo. The other species were classified as mesothermic or megathermic. The latter show preference for tropical areas: among them are those widespread in the neotropics, those found all over South America, and those from central or northeastern Brazil, with their southern limits in São Paulo. The group of mesothermic species includes exclusively grassland herbs, with almost all populations below the Tropic of Capricorn, and northern limit in São Paulo State. The bearing of these data on neotropical biogeography is stressed.

P1131. A taxonomic revision on the genus of *Euphorbia* (Euphorbiaceae) in Iran

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Euphorbiaceae is the fifth great family of flowering plants and *Euphorbia* is one of the largest genus of flowering plants that based on Flora Iranica consists of over 100 species in Iranica region and more than 60 species in different parts of Iran. In this work plants belong to this genus were studied and in this order to all of the herbarium materials preserved in a few herbaria collected from different parts of Iran and specimens in the field were studied and determined and the following results are presented:

- There are 65 species of *Euphorbia* in different parts of Iran.
- The species *E. rosularis* A. THEOD. and *E. maculata* L. are recorded for the first time from Iranica area and the flora of Iran. These species in the flora of USSR. have been formerly known as an endemic of Turkmenistan and caucasus respectively.
- The species *E. franchetii* B. FEDTSCH., *E. grossheimii* PROKH., *E. consanguinea* SCHRENK and *E. kopetdaghi* PROKH. are reported for the first time for the Flora of Iran

- *E. cheirolepioides* RECH.f. is synonym with *E. grossheimii*.

-The species *E. aellenii* RECH.f. according to this study is synonym with *E. kopetdaghi* PROKH..

P1132. Character evolution of *Alnus* (Betulaceae) and fossil leaves and cones from the Tertiary of Northern Thailand

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Alnus Mill. (Betulaceae) today comprises approximately 35 species and is widespread in the temperate Northern Hemisphere, extending to Southeastern Asia and to the Andes. Tertiary macrofossils are widely distributed in North America, Europe, and Asia, usually as isolated leaves and infructescences. Recent leaves were surveyed to search for taxonomically important characters to allow placement of fossil leaves within subgenera or smaller subtaxa of *Alnus*. Semicraspedodromy was observed in some species of the subgenera *Alnus* and *Clethropsis*, but not in *Alnobetula*. Character evolution analysis using parsimony suggested that craspedodromy was plesiomorphic in the genus with independent evolution of semicraspedodromy in three subclades. Veins reaching the sinus before branching to the teeth is a derived character having evolved one or more times. Fossil leaves and seed cones were found in lacustrine deposits in early Miocene or late Oligocene basins in Northern Thailand. The venation pattern and teeth of the leaves are most similar to those of Recent *Alnus fernandi-coburgii* and *A. cremastogyne* (both subgenus *Alnus*) from China.

P1133. Cuticle micromorphology and anatomical structures of leaves of *Fagus* L. (Fagaceae) and its taxonomic implication

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The genus *Fagus* is distributed over the Eastern Asia, U.S.A. and Ukraine in the northern hemisphere. Many kinds of taxonomic system have been controversial. Cuticle micromorphology and anatomical structure of all 8 species of *Fagus* and outgroup were examined. 23 cuticle morphology have been described. The subsidiary cell shape, size of stomata, and so on, are considered important characters for the infrageneric classification. In the anatomical structure studies, 13 characters of anatomical features have been described. Shape of epidermal cells, papillae and shape of vascular bundle are considered important. A parsimony analysis of 26 characters resulted in a single most parsimonious tree with consistency indices of 0.73 and retention indices of 0.73 and tree lengths of 48 steps. The topology obtained from the analysis showed two major clade. The first clade was supported bootstrap value 80%, and the second clade bootstrap value 87%. Based on the cuticle morphology, the taxonomic system of Shen(1992) was generally supported except *F. longipetiolata*.

P1134. Alders (*Alnus* Mill., Betulaceae) in the European Russia

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Alders (*Alnus* Mill.) are the common trees in Europe and Asia, and there are some taxonomically interesting forms on the border of the area. The examples of those forms are *A. kolaensis* Orlova (from Kola Peninsula and nearby North Karelia) and *A. barbata* C.A. Mey. (from Caucasus). Some researchers consider these forms as separate species, but others -- as subspecies of *A. incana* or *A. glutinosa* (respectively), or even as hybrids between these two species. We have checked mentioned hypotheses using multivariate methods based on classic morphometrics data and two variants of geometric morphometrics of alder leaf. We found that *A. "kolaensis"* is most probably the varieties or even the ecological form of *A. incana*. On the contrary, *A. barbata* must be considered as separate species. Our data show that the results of geometric morphometrics and classic morphometrics could be significantly different. The most productive way of placing the landmarks on the leaf is (in our case) to mark the end-points of the secondary veins.

P1135. Cuticle micromorphology of leaves of *Quercus* L. (Fagaceae) and its taxonomic implicationJ. H. Pak¹, S. H. Cho¹, K. J. Kim²;¹Kyungpook National University, Daegu, Republic of Korea, ²Graduate School of Biotechnology, Seoul, Republic of Korea.

The genus *Quercus* is widespread in the Northern hemisphere in habitats ranging from temperate and tropical forests to dry thorn scrub and semi-desert. Camus recognized 2 subgenera and 6 sections. However, some taxonomic systems are still different from each other. Cuticle micromorphology of 22 species of *Quercus* and outgroup were examined by the SEM. 22 species selected each two or more species in all section of genus *Quercus*. Outgroup selected the genus *Trigonobalanus* and *Alnus* (Betulaceae). 10 characters of the inner surface and 8 characters of the outer surface of the cuticle have been described. The presence of papillae, arrangement of subsidiary cell, shape of anticlinal cell wall are considered important characters for infrageneric classification. A parsimony analysis of 18 characters resulted in 72 most parsimonious trees with tree length of 66 steps. The topology obtained from the analysis showed 2 major clades. Based on the cuticle morphology, the two subgenus delimitation of Camus was supported. However, sect. *Erythrobalanus* and sect. *Cerris* formed one group, and sect. *Lepidobalanus* formed polytomy.

P1137. Inflorescence and floral development in *Streptocarpus* and *Saintpaulia* (Gesneriaceae)

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Floral development and inflorescence structure within *Streptocarpus* and *Saintpaulia* were investigated using Scanning Electron Microscopy (SEM). We discuss the structure of the pair-flowered cyme and the floral ontogeny found in the Gesneriaceae in a phylogenetic context with particular reference to an East African clade of *Streptocarpus* and *Saintpaulia*. Current phylogenetic hypotheses divide the caulescent East African *Streptocarpus* species into two distinct clades, one of which (*Str. caulescens*, *Str. glandulosissimus*, *Str. holstii* and *Str. buchananii*) is sister to a monophyletic *Saintpaulia* clade. In both clades of caulescent East African *Streptocarpus*, helical sepal initiation was observed, as opposed to the apparently more general adaxial to abaxial unidirectional pattern in *Streptocarpus*. In the *Str. caulescens* clade bracteoles were absent or present on only one lateral axis, with a monochasial branching pattern in which the lateral cyme unit develops on the ebracteolate axis. The impact of bracteole loss on the developmental stages of the inflorescence was evaluated.

P1138. Molecular identification of *Aeschynanthus* (Gesneriaceae) leading to a discovery of an undescribed speciesJ. Denduangboripant¹, P. Jaidee², W. Chulalaksananukul², S. Panha¹;¹Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, ²Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

Aeschynanthus is an epiphyte genus widespread in SE Asia. Species identification of the genus relies heavily on their flower and seed morphology. This usually causes a great difficulty when the reproductive parts are unavailable. We therefore performed molecular identification using our previously-prepared *Aeschynanthus* phylogeny based on internal transcribed spacer (ITS) sequences. Several cultivated plants were bought and their ITS sequences were obtained. One plant was suggested to be *Columnea* sp., misunderstood by a plant seller to be *Aeschynanthus*. Others were proposed to be *A. radicans*. We also collected some wild *Aeschynanthus* seedlings which then were genetically suggested to be *A. hildebrandii*. Interestingly, one plant sample with very unusual pinkish flowers showed unique ITS sequence characteristics and may putatively be an undescribed species. This molecular identification study of cultivated and wild *Aeschynanthus* therefore showed a great advantage of using molecular techniques to identify any problematic taxon, especially when it is difficult to investigate taxonomically significant organs.

P1139. Reproductive biology of African violet (*Saintpaulia*): evolutionary and conservation consequencesJ. K. Kolehmainen¹, P. Mutikainen²;¹University of Helsinki, Helsinki, Finland, ²University of Oulu, Oulu, Finland.

To find out the reproductive potential and evolutionary mechanisms affecting species integrity and population viability of the endangered genus *Saintpaulia*, flower and seed biology, flowering phenology and pollination of three *Saintpaulia* species were studied in the East Usambara Mts., Tanzania. The synchrony observed in flowering in *S. confusa* and *S. difficilis* may enable hybridization between these two species, but partial phenological separation may contribute to the integrity of *S. grotei*. Although the level of flower abortion is high in *S. confusa*, each pollinated flower yields about 1000 seeds, a large proportion of which is probably deposited in the seed bank. Seed germination level is also high. *S. confusa* produces fruits following both self and cross pollination whereas spontaneous self pollination seems not to occur. Thus, seed production depends on sufficient pollinator service. Floral heteromorphy (i.e. enantiostyly) likely further enhances cross pollination, suggesting that the genus predominantly outcrosses. Thus, *Saintpaulia* populations are likely to suffer from negative effects of inbreeding if they become small and isolated.

P1140. Molecular origin of floral symmetry and inflorescence morphology in GesneriaceaeC. Wang^{1,2}, M. Möller³, Q. C. B. Cronk²;¹Institute of Ecology and Evolution, Taipei, Taiwan Republic of China, ²Botanical Garden and Centre for Plant Research, University of British Columbia, Vancouver, BC, Canada, ³Royal Botanic Garden Edinburgh, Edinburgh, United Kingdom.

The GCYC genes are involved in the development of zygomorphy in flowers. We have compared GCYC expression between wild type and the peloric form (actinomorphy) of African violet. Both GCYC homologues (*SaGCYC 1A* & *SaGCYC 1B*) of the wild type *Saintpaulia* are expressed only in the adaxial (dorsal) petals and sepal, and *1B* is more strongly expressed than *1A*. Surprisingly, *SaGCYC 1A* & *1B* are also expressed only dorsally in the flower of the peloric form, with an even higher expression level. The increased *1A* & *1B* transcript level implies that the peloric mutation is in a gene downstream of GCYC, involved in regulatory feedback of GCYC. The ventral identity gene *DIV* is a possible candidate. We also isolated the inflorescence identity gene *GFLO* from *Titanotrichum* to study its effect on the development of the inflorescence. The *GFLO* expression pattern indicates that it is required for inflorescence initiation and flower induction. However, the *GFLO* transcript is reduced when bulbiferous shoots start to initiate. The *GFLO* down-regulation appears to correlate with the loss of floral determinacy in the raceme inflorescence, and therefore with the generation of side branches.

P1141. Phylogeographic notes of Gesneriaceae in the Ryukyus, JapanG. Kokubugata¹, M. Yokota², S. Kobayashi³, C. Peng⁴;¹National Science Museum, Tokyo, Tsukuba, Ibaraki, Japan, ²University of the Ryukyus, Nishihara, Okinawa, Japan, ³Makino Botanical Garden, Kochi, Japan, ⁴Academia Sinica, Taipei, Nankang, Taipei, Taiwan Republic of China.

The family Gesneriaceae consists of ca. 3,700 species in 147 genera that are distributed mainly in the tropics with some members extending into temperate zones. Seven species are known from the Ryukyus islands that are situated between Kyushu (Japan) and Taiwan. *Aeschynanthus acuminatus*, a widely distributed species in Asia, and has been previously documented only once from Iriomote island of the Ryukyus (in 1973). We (SK) reconfirmed the presence of this locally rare species on this island in 2004. *Conandron ramondioides* occurs in China, Taiwan and Japan (including Iriomote). Taxonomic treatments for this widespread but scattered species have been varied. Based mainly on corolla morphology, we concluded that plants from the Iriomote and Taiwan were distinguishable from those of mainland Japan. *Titanotrichum oldhamii* is distributed in China, the Ryukyus and Taiwan. In the Ryukyus, this species are known from Ishigaki and Iriomote islands. However, little has been known of this species in Ishigaki. We (MY) documented a new habitat of *T. oldhamii* on this island in 2004.

P1142. The developmental analyses of the unique leaf morphogenesis in rosulate *Streptocarpus*

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In common dicots, leaves are formed from shoot apical meristem (SAM) mostly of a tunica-carpus structure. However, acaulescent species of *Streptocarpus* form leaves without SAM. We are interested in elucidating this unique leaf formation process, and have performed developmental analyses. When we followed the leaf formation pattern, we found 1st leaf was always observed at the axil of macrocotyledon where caulescent species retain SAM. As it was revealed that 1st leaf was formed consistently at this place, we could follow this process in detail. When leaf promordium bulged, the tunica-carpus-like structure that was previously identified as groove meristem (GM) was observed, and differentiated to leaf. Although it was said by previous other studies that cells in the GM divide to form a leaf by themselves, it was revealed that tissues of inner parenchyma and procambium in the petiolode tissue adjacent to the GM divided to the formation of 1st leaf actively as well as the GM did. Thus it is inferred that the GM would have a role in forming leaves as a kind of leaf organization center. These outcomes are discussed in reference to internal factors that affected these processes.

P1143. Developmental and genetic aspects of the actinomorphic flowers of *Bournea* (Gesneriaceae)

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Bournea Oliv. is one of the few genera of Gesneriaceae having actinomorphic flowers. Regarding its two species, *B. leiophylla* has pentamerous flowers, while those of *B. sinensis* are tetramerous. Floral development of *B. leiophylla* shows that even though five stamens are basically equal in length in the mature flower, the adaxial and lateral stamens are remarkably smaller than the two abaxial stamens during early developmental stages. Four developmental genes associated with floral symmetry have been isolated from the floral tissues in *B. leiophylla* and *B. sinensis*. The four genes include *GDIV1* and *GDIV2*, two homologues of the *DIVARICATA* gene (*DIV*) in the model plant *Antirrhinum majus*, and two *CYCLOIDEA*-like genes (*CYC*), *GCYC1c* and *GCYC1d*. Remarkable are 40-80bp insertions both in *GCYC1c* and *GCYC1d* of *B. sinensis*. Perhaps they are possibly responsible for the fusion of the two adaxial petals of *B. leiophylla* into a single adaxial petal in *B. sinensis*. Comparison of expression of the four developmental genes suggest a network in function and regulation of these genes associated with the origin and developmental evolution of the actinomorphic flowers in *Bournea*.

P1144. Molecular phylogenetic studies and character evolution of *Didymocarpus* Wall. from Thailand

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Molecular phylogenies have been generated to investigate relationships among species of *Didymocarpus* Wall. in Thailand. Fifteen Thai species have been included in a parsimony analysis using the internal transcribed spacer (ITS) of ribosomal DNA (rDNA). To test the relationships among the Thai species, additionally, four species from China, three species from Malaysia and one species from Bhutan were also included, as well as five outgroup taxa, three *Chirita* spp., *Briggsia muscicola* and *Oreocharis auricula*. The trees were rooted on the latter. The analysis show that the *Didymocarpus* species included formed a strongly supported monophyletic clade. The genus included two strongly supported clades. The results further suggest that the origin of *Didymocarpus* is the Malay Peninsula with whitish to yellow corolla, followed by species from southern and eastern Thailand, China and Bhutan with a corolla shape reduced to a narrow tube of purple to violet. To fully understand the evolution in this genus inclusion of further material is required.

P1145. Gentianaceae in the flora of Ukraine

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As a result of our taxonomical revision of Gentianaceae family in the flora of Ukraine, the systematical structure was specified. In the Ukrainian flora this family is represented by *Blackstonia* (*B. acuminata* (W.D.J.Koch & Ziz) Domin, *B. perfoliata* (L.) Huds.), *Centaurium* (*C. erythraea* Rafn, *C. littorale* (D.Turner) Gilmour, *C. pulchellum* (Sw.) Druce and 2 subspecies, *C. spicatum* (L.) Fritsch, *C. uliginosum* (Waldst. & Kit.) G.Beck ex Ronniger), *Gentiana* (*G. acaulis* L., *G. asclepiadea* L., *G. cruciata* L., *G. laciniata* Kit. ex Kanitz, *G. lutea* L., *G. nivalis* L., *G. pneumonanthe* L., *G. punctata* L., *G. utriculosa* L., *G. verna* L.), *Gentianella* (*G. amarella* (L.) B. mer and 2 subspecies, *G. lutescens* (Velen.) Holub and 2 subspecies), *Gentianopsis* (*G. ciliata* (L.) Ma Yu-Chuan) and *Swertia* (*S. alpestris* Baumg., *S. perennis* L., *S. punctata* Baumg.). Occurrence of *Gentiana frigida* Haenke and *G. tenella* L. was not confirmed in the Ukrainian flora. Meanwhile, we propose to include *Gentiana acaulis*, *G. laciniata*, *G. lutea*, *G. nivalis*, *G. punctata*, *G. utriculosa*, *G. verna*, *Swertia alpestris* and *S. perennis* into the new version of "The Red Book of the Ukraine".

P1146. Problems with Gentianaceae in regional floras in Brazil

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Gentianaceae are represented in Brazil by c. 28 genera and 90 species, belonging to three of the five tribes of the family: Saccifolieae (*Curtia*, *Hockinia*, *Saccifolium*, *Tapeinostemon* and *Voyriella*), Chironieae (*Centaurium*, *Coutoubea*, *Deianira*, *Schultesia*, *Symphylophyton* and *Zygostigma*), Helieae (*Adenolisianthus*, *Aripuana*, *Calolisianthus*, *Celiantha*, *Chelonanthus*, and *Helia*) and *Voyria*, a genus still *incertae sedis*. The only flora dealing with the family as a whole is the Flora brasiliensis in the 19th century. More recently appeared the floras of Rio de Janeiro (1966) and States of Santa Catarina (1971) and São Paulo (2005). In the mountains of central Brazil, the Floras of the Serra do Cipó (1987) and Grão Mogol (2004) both in the State of Minas Gerais and the Pico das Almas in the State of Bahia (1995) were produced. In the north, the family was treated in the Floras of Guayana Highland (1978) and of the Reserva Ducke (1999) in the State of Amazonas. Since only few revisions are published and many of the species are narrow endemics and poorly known, there are problems on species circumscriptions. Generic limits are likewise controversial, mainly in tribe Helieae.

P1147. Phylogeny of *Gentiana* Sect. *Calathianae* based on chloroplast PCR-RFLP

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Gentiana Sect. *Calathianae* is distributed in Europe, Asia, North America and North Africa, primarily occupying mountainous to alpine habitats. A phylogeny based on chloroplast PCR-RFLP is presented, including 18 taxa with 250 accessions, using 8 different non-coding regions, each digested with 4 enzymes covering about 15% of the plastid genome. Different monophyletic lineages can be recognised, such as the two annuals *G. nivalis* and *G. utriculosa* and the eastern Alpine *G. pumila* s.str. that is separate from *G. pumila* subsp. *delphinensis*. The taxa of *G. verna* s.l. are divided into an Alpine/Pyrenean clade and another covering the southeastern European mountains. *G. brachyphylla* s.str. from Alpine populations is placed in a separate lineage where it groups neither with *G. brachyphylla* subsp. *favratii* nor with plants from southern Spain. *G. bavarica* and *G. rostani* represent two distinct monophyletic species placed in different subclades.

P1148. Seed and pollen morphology of the Neotropical moon-gentians (Macrocarpaea: Gentianaceae)

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There are many taxonomically useful seed morphological characters in Macrocarpaea. Therefore, seeds of 81 species (out of 106 in the genus) were studied by SEM. Mature seeds are minuscule in size, 0.2-2.2 x 0.2-1.0(-2.2) mm, yet display a wide range of diversity. Four broad morphological seed types are identified, conforming to the four sections of the genus: "Flattened type seeds" for sect. Tabacifoliae (southeastern Brazil), "Perimetrically winged type seeds" for sect. Choriophylla (central Andes, especially the Huancabamba region), "Rimmed type seeds" for sect. Macrocarpaea (northern Andes, southern Mesoamerica, Greater Antilles, and Guayana Shield), and "Winged

type seeds" for sect. *Magnolifoliae* (central Andes). Additionally, SEM studies of pollen exine in 88 species confirm the presence of two morphological types. The *Glabra*-type (3-colporate, spheroidal with reticulate exine) is present in most species of the genus, while the *Corymbosa*-type (formerly *Rusbyanthus*-type) (3-colporate, spheroidal, with warty exine) occurs in 13 species of sect. *Magnolifoliae* providing an additional unique character to define the section.

P1149. SEM observations of seeds of gen. *Gentiana* L. and their systematic significance

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The genus *Gentiana* includes about 360 species distributed in temperate, arctic and alpine parts of the northern hemisphere mainly. Seeds of gentians show high diversity in size, shape and surface pattern, and furthermore have been reported to be of systematic importance in this genus. Unfortunately, until now, seeds of only a limited number of taxa have been studied in detail. As part of a taxonomic study of the genus *Gentiana* we investigated seed morphology in order to evaluate its taxonomic significance.

Seeds of taxa from 7 sections (*Pneumonanthe*, *Cruciata*, *Frigida*, *Isomera*, *Microsperma*, *Kudoa*, *Monopodiae*) were examined with the scanning electron microscope. Qualitative characters of seed shape and testa ornamentation were selected and cluster analysis (UPGMA) was performed on the base of presence and absence of coded characters.

Based on seed shape, testa cell shape, and surface ornamentation, eight morphological types are recognized. The results of cluster analysis revealed that seed micromorphology follows the sectional classification, thus providing a useful tool for studies on evolution and systematics of gentians.

P1150. The Indo-Chinese Gentianaceae

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Introduced in 1789 by Jussieu, the Gentianaceae gathered about 10 genera. Later, other scientists attached to this family a great deal of species and genera, spread in various tribes.

Presently the Gentianaceae accounts ca. 76 genera and 100 species. Cosmopolitan, it mostly spreads upon temperate areas in either high or low altitude; in tropical Asia it is encountered in wet district of mountains, sometime in savannah or in clear deciduous woods with Dipterocarpaceae. Dop & Gagnepain mentioned 19 genera and 22 species in Cambodia, Laos and Vietnam. Following our study 10 genera and 27 species were listed to the Flora of Cambodia, Laos and Vietnam.

While revising Gentianaceae for this Flora, the study of material from Asia, mostly the Indo-Chinese Peninsula drove us to identify 40 species. Among these species, 11 new species and 1 new subspecies are recorded.

P1151. Conflicting genetic and morphological patterns in European *Gentianella* sect. *Gentianella*

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The low divergence in ITS nrDNA and *trnL*-F cpDNA sequences indicates a very young diversification within European *Gentianella* Moench sect. *Gentianella*. AFLP data, however, revealed strong differentiation on the geographical scale despite low morphological differences. In contrast high genetic similarities were found among taxa of the Alps, that are morphologically very distinct. In a morphological analysis we found only few morphological characters of taxonomic significance. In one of these characters: papillae on the calyx lobes, we demonstrated by Scanning Electronic Microscopy (SEM) that there are three different types that were not distinguished hitherto. A strong response to the environmental variable altitude was found in nearly all morphometric traits. Altogether genetics and morphology show different patterns in the sect. *Gentianella* which raises questions on morphological homoplasy and reticulate evolution.

P1152. Testing the monophyly of selected infrageneric taxa within the genus *Mimosa* (Leguminosae, Mimosoideae)

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Mimosa is the largest genus in tribe *Mimoseae* (510 species) occurring mainly in America. The aims of this study are: 1) to test the monophyly of sections of *Mimosa*; 2) to test the monophyly of three series of sect. *Batocaulon*; 3) to assess the phylogenetic relationship among the New World species and allied Old World ones, and 4) to test the sister group relationship between *Piptadenia* and *Mimosa*. This phylogenetic analysis, based on morphological, palynological, and anatomical data, was carried out using PAUP and Winclada; species of *Mimosa* (twenty-seven), *Piptadenia* (four), and one species each of *Pithecellobium*, *Enterolobium*, and *Acacia*, were studied. Fifty-five unordered characters were scored, with equal weighting. Our conclusions are: 1) Monophyly of *Mimosa* is confirmed. Section *Calothamnus* is monophyletic, whereas *Mimadenia*, *Batocaulon*, *Habbasia*, and *Mimosa* are not; 2) Series *Leucaenoideae* and *Rubicuales* are not monophyletic; ser. *Bahamenses* groups with Mexican and Madagascan species; 3) *Piptadenia* is not monophyletic and, in part, is sister to *Mimosa*; 4) Our results are the basis to carry out a collaborative, molecular phylogenetic study of *Mimosa*.

P1153. Taxonomy of *Astragalus* Subgenus *Cercidothrix* (Fabaceae) in Iran: Complex *Dissitiflori*

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Astragalus is the biggest genus of flowering plants with nearly 2500 species.

This paper is about a part of bifurcating hairy *Astragalus* sections. Bifurcating hairy *Astragalus* are involved in the *Astragalus* subgenus *Cercidothrix* Bunge, which is characterised by the presence of bifurcate hairs. This subgenus has nearly 850 species in the Old World, nearly 200 of which occur in Iran. Iranian species occur in less than 20 sections.

The subject of this paper is taxonomy of six related and nearly homogenous sections in these subgenus. Four sections are more herbal: Sect. *Craccina* (Steven) Bunge (24 species), Sect. *Hololeuce* Bunge (36 species), Sect. *Onobrychoidei* DC. (83 species), and Sect. *Ornithopodium* Bunge (17 species). Two other sections are more woody sections: Sect. *Erioceras* Bunge (37 species), and Sect. *Dissitiflori* DC (187 species).

Here these sections are studied morphologically and anatomically. Based on these study the most important characteristics for the separation of the sections, relationships between the sections and identification key for them are presented.

Here it is revealed these are natural groups.

P1154. Remarks on *Medicago* sect. *Dendrotelis* (Fabaceae) based on morphological and molecular data (ITS and *trnL*-F)

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Medicago sect. *Dendrotelis* includes shrubby perennial plants with stipitate pods with 1-3 coils, flowers with the keel equaling or slightly longer than the standard, and embryos with the radicle about half as long as seed. Three Mediterranean taxa native to littoral areas and islands are included: *M. citrina*, *M. arborea* and *M. strasseri*. The taxonomic status of *M. citrina* varies depending on different criteria. To clarify this point, macromorphological (leaves, petals, pollen, pod, etc.) and micromorphological data (pollen, leaf, etc.), together with sequences of two DNA regions (nuclear ITS, internal transcribed region; and the plastid *trnL*-F region (the *trnL* intron and the *trnL*-*trnF* intergenic spacer) were studied. The combined data showed sect. *Dendrotelis* as a monophyletic group, related to sect. *Medicago*. Within this clade, the western Mediterranean *M. citrina* appears as the sister group of the eastern taxa (*M. arborea* and *M. strasseri*).

P1155. Comparative anatomy of seed coats with some characteristic patterns on their surface in subfamily Caesalpinioideae (Leguminosae)

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Seed surface patterns such as pleurogram-areole and pitted ones were anatomically investigated and compared in the subfamily Caesalpinioideae. The seed coat is generally composed of cuticle, epidermal palisade cells, hypodermal hour-grass cells, and parenchyma cells. The epidermal palisade cells are separated into two layers by a light line running across each cell periclinally, and the relative height of the two layers varies among species and also partly within a seed. In the latter cases the conspicuous variation in the relative height is found between the inside and outside of pleurogram. Based on variation in the relative height the pleurograms are distinguished into two types in the Caesalpinioideae, one of which is similar to those found in the subfamily Mimosoideae. This type occurs in genera of Dimorphandra group of the tribe Caesalpinieae, which is located on the closest position to the Mimosoideae. In *Senna*, moreover, the areole was anatomically shown to be similar to that of the whole seed of the species without pleurogram-areole pattern. Although *Chamaecrista* has the peculiar pitted pattern, the seed coats are similar to those of the areole of *Senna*.

P1156. Understanding diversification of Neotropical *Acacia* (Leguminosae: Mimosoideae) and estimating endemism indices.

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Acacia, the second largest genus in the family Leguminosae, is currently a pantropical genus with 1 350 species placed into three subgenera. Most species are native to Australia where subgenus *Phylodineae* is predominant. Maslin (2003) presented a comprehensive historical review of the generic status. American *Acacia* comprises c 158 species with c. 49 infraspecific taxa. Subgenus *Aculeiferum* section *Filicinae* (c 20 taxa) is excluded from *Acacia* and placed in *Acaciella* Britton & Rose. In the neotropics subgenus *Aculeiferum* comprises 98 species, 34 vars. and subgenus *Acacia* 60 species, 17 vars. Ecologically, *Acacia* is mainly associated with drylands and secondary growth, but numerous species are elements of tropical wet forest. A component analysis of the species was undertaken using c 50 different geographical regions to estimate endemism indices. Analysis of morphological patterns was based mainly on inflorescence, fruit and seed structure. These analyses show that the most specialised group (myrmecophyllous species) is concentrated in the Mesoamerican region. A table of species habitats and conservation status is presented.

P1157. Molecular phylogeny of *Andira* Lam. nom.cons. (Leguminosae, Papilionoideae): A case study of neotropical speciation

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The nuclear ribosomal internal transcribed spacer region (ITS) was sequenced for 42 accessions representing 25 of the 29 species of *Andira* (Leguminosae, Papilionoideae). Maximum parsimony, maximum likelihood and Bayesian analyses produced similar tree topologies, demonstrating the monophyly of *Andira* and resolving two major clades. Relationships among five, small lineages remain largely unresolved and poorly supported. Molecular rates analysis suggests multiple Amazonian rain forest lineages, two Guayanan rain forest lineages, and a Brazilian Atlantic coastal forest lineage diverged in the Miocene. Movement out of the rain forest habitat into new biomes (seasonally dry tropical forest, cerrado, montane forest, restinga) accompanied bursts of speciation across the genus in the Plio-Pleistocene with as many as twelve *Andira* species originating since that time. *Andira* demonstrates an evolutionary history of both ancient and recent speciation.

P1158. Evolutionary history of the highly diverse Genistoid core (Leguminosae) inferred from phylogenetic analyses of nuclear ribosomal (ITS and ETS) and plastid (*trnL-trnF* IGS) DNA sequences

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Phylogenetic analysis of the Genisteeae s.l. recognises a well-supported genistoid core, comprising *Cytisus*- and *Genista*-related genera (the 'Cytisus group' and 'Genista group'), and several other taxa whose relationships are not yet well established (*Lupinus*, *Argyrolobium*, *Adenocarpus*). We present phylogenetic trees based on ITS and ETS regions (nrDNA), and *trnL-trnF* IGS (cpDNA) of the genistoid core. The nrDNA regions provide more parsimony informative characters than the cpDNA, the latter shows indels and rearrangements that yields relevant phylogenetic information. A comparison of the topologies provided by the different markers shows incongruence/instability in the position of key taxa (e.g. *Argyrocytissus*, *Erinacea*, *Gonocytissus*, *Petteria Retama*, *Spartium*) that is suggestive of reticulate evolution. We also discuss the evolutionary pattern differences of the *Cytisus* group vs the *Genista* group, and the position of small segregate genera (e.g. *Podocytissus*, *Lembotropis*, *Pterospartum*).

P1159. Critical taxonomical revision of Fabaceae in Ukraine flora.

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Behind the different data the family Fabaceae of Ukraine flora totals from 300 up to 500 species. Such significant distinction among indicated species is caused various by treatments taxonomical volume of some taxa of a species rank and genera. It causes significant different interpretations in comparison with floras of neighbouring states and territories. The critical taxonomical revision of the family will be spent with the purpose of the coordination of nomenclature and taxonomical disagreements in frameworks of Central European flora. The plenty of species rank taxa was described earlier wrongly in genera *Astragalus*, *Medicago*, *Lotus*, *Genista* etc. The critical analysis of the chorological information on presence of those or other species in territory of Ukraine is necessary, as many species were specified for territory wrongly. The important meaning for this purpose there is a critical processing of herbarium materials, which were a little involved in time of taxonomical processings of the family in last 40 years. On tentative estimations the aggregate number of species of the family will make about 400 taxa of a species rank.

P1160. Phylogenetic analyses of subtribe Trigonellinae: *Medicago*, *Melilotus*, and *Trigonella* (Leguminosae) using nucleotide sequence data from two nuclear protein-coding genes, *Le* and *pgiC*, and plastid gene *matK*

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Phylogenetic relationships of species in subtribe Trigonellinae (*Medicago* and *Trigonella*) were examined based on analyses of nucleotide sequences of the plastid gene *matK* and two nuclear genes, Mendel's stem length gene, *Le*, and the gene encoding cytosolic phosphoglucose isomerase, *pgiC*. Perennial species of *Trigonella* section *Ellipticae* that had not been included in previous molecular analyses were also sampled. Relationships of species of *Medicago* previously included within *Trigonella* and species of section *Medicago* (including *Medicago sativa*) were of particular interest. Results of the analyses were used to evaluate characteristics of the legume that have been considered important in past generic delimitation, but are subject to some obvious as well as more subtle convergent evolution. Species in subtribe Trigonellinae include a geocarpic annual, annual and perennial herbs, and shrubby species; this variation in habit is also considered in a phylogenetic context.

P1161. Molecular phylogenetics of *Amorpha* L. (Fabaceae) based on evidence from chloroplast and nuclear markers

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Amorpha L. is a genus of about 15 species belonging to the tribe Amorpeae within the papilionoid subfamily of legumes. The taxonomy of this group is complex and perplexing due to the high degree of environmental plasticity exhibited within species and gradation of character variation between species. This genus has not previously been the subject of phylogenetic analysis based on either morphological or molecular characters. The complicated taxonomic history of *Amorpha* makes the use of molecular phylogenetic analyses an appropriate measure to resolve relationships. Ascertaining the origin of the constituent genomes of *Amorpha fruticosa*, a tetraploid, is one goal of this study. A number of chloroplast haplotypes based on spacer region sequences have been observed within and between species. In addition, many low copy nuclear candidate genes containing introns have been identified through comparison of *Medicago* and *Arabidopsis* sequences. These include *ARG1* and *sterol 24-C-methyltransferase*, which show intron variation between taxa. Phylogenies of these and other unlinked markers will allow analysis of sources of incongruence among *Amorpha* species.

P1162. Phylogenetic relationships among species of *Mimosa* (Leguminosae) based on cpDNA.

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Phylogenetic relationships among 26 southamerican species of genus *Mimosa* were analysed on the basis of both the intron and the intergenic spacer TrnL-TrnF in order to clarify taxonomic assignments based on morphological characters. Seven species representing related genera were also sequenced and analyzed. The basic data matrix included 1137 characters. A total of 144 equally parsimonious trees were retrieved (633 steps, IC=0.66 and IR=0.59). The consensus tree showed that *Mimosa* might be a monophyletic group sister to genus *Piptadenia*. These preliminary results indicate that two groups can be distinguished within *Mimosa*: one of them includes species of the section *Baulocaulon* DC. and the other one species of the sections *Habbasia* DC., *Mimosa* and *Caulothanmus* Barneby. The latter might be sister to *Scrankia*, now recognized in the synonymy of *Mimosa*. Finally, the species of the section *Caulothanmus* studied here did not group within the same clade suggesting that this section is not a natural group.

P1163. Is there congruency between traditional classification and molecular relationships in the genus *Cicer* L.? Insights from nuclear and chloroplast sequence data

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Cicer L. is a genus composed of ca. 43 species with distribution in Central and Western Asia, Europe and Africa. Four sections have traditionally been defined based on morphological characters and geographical distribution: *Cicer*, *Chamaecicer*, *Polycicer* and *Acanthocicer*. As a part of comprehensive systematic study of the genus *Cicer*, nuclear ITS and chloroplast *trnK/matK* sequence data were determined to elucidate the congruence between traditional classification and molecular relationships in the genus. Twenty-eight species belong to four sections and five outgroup taxa from *Vicieae*, *Trifolieae*, and *Galegeae* were analyzed in this study. The maximum parsimony analyses of nrDNA and cpDNA data indicate three major clades and non-monophyly of the annual species in the genus *Cicer*. Molecular data suggest that traditionally defining features are actually phylogenetically homoplastic, leaving present infrageneric boundaries in doubt and a taxonomic realignment of infrageneric delimitation and composition should be considered. The character evolution of life cycle, pollen morphology, and canavanine content will be discussed in the phylogenetic tree.

P1164. Floral organogenesis in tropical legume genera (*Adesmia* and *Aeschynomene*) and its systematic implications.

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Floral organogenesis of *Aeschynomene* (*A. falcata* and *A. sensitiva*) and *Adesmia* species (*A. securigerifolia*, *A. riograndensis*, *A. punctata* var. *hilariana*, *A. latifolia*, *A. ciliata*, *A. tristis* and *A. muricata*) were studied using scanning electron microscopy. The aims were to identify ontogenetic characters and discuss its systematic value. In *Aeschynomene* species the five isolated sepals primordia are initiated in unidirectional order from abaxial side. Otherwise, in *A. sensitiva* these primordia were grouped in two poles forming a two-lobed calyx and petals initiate asymmetrically. This and others ontogenetic differences corroborates with the separation of section *Ochopodium* from *Aeschynomene* subtribe. The *Adesmia* species share the initiation of sepal whorl in unidirectional order from adaxial side, except in *Adesmia latifolia*, that initiates in simultaneous way. This ontogenetic character was considered genera diagnostic because is completely different from other papilionoids. Others ontogenetic differences between *Adesmia* species were described, and infrageneric taxa reorganization was suggested.

P1165. Phylogeny and systematics of the genus *Lathyrus* L. (Fabaceae): evidence from nuclear ITS sequences

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The genus *Lathyrus* (Fabaceae) comprises c. 160 species of annual and perennial herbs, morphologically and ecologically diverse, with a worldwide distribution. Due to their resistance to drought and flooding, some *Lathyrus* species (e.g. *L. sativus* - grass pea - and *L. cicera* - chickling vetch) have great agronomic potential as supplementary sources of calories and protein for human populations and as forage crops in the poorest regions in the World. The systematics and phylogeny of the genus have been investigated using both morphology and molecular data, mostly plastid DNA. We used the nrDNA ITS region to investigate the relationships in the genus, including a representative sampling of its sections and main clades, as well as species from other genera in the tribe *Vicieae*. *Lathyrus* and *Pisum* are shown as a strongly supported monophyletic group. Phylogenetic resolution within *Lathyrus* is still low, but it might be improved with the increasing sampling. Nevertheless, various groups are recognized, some confirming previously suggested associations, such as the affinity of Sect. *Aphaca* to Sect. *Pratensis*, and the close relationship of *L. clymenum* and *L. ochrus*.

P1166. Phylogenetic Relationships in Iranian *Medicago* Species Using RAPD Markers

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Genetic diversity of 54 populations from 22 species of *Medicago* collected from Iranian natural habitat was studied. DNA was extracted from bulked leaf samples of each population and used for RAPD. RAPD markers produced by 11 UBC primers was analyzed according to Maximum Parsimony method. Using PAUP software, a phylogenetic tree with 5 main clusters was generated. Populations of *M. aculeata*, *M. constricta*, *M. rigiduloides* and *M. rigidula* with hard pod walls and spongy texture were classified in one cluster. Populations of *M. sauvagei*, *M. laciniata* and *M. polymorpha* with soft and flexible pod walls were classified in a separate cluster. Populations of each species were classified in subclusters, indicating that RAPD was capable of identifying genetic diversity at the species level. Populations of one species grown under similar climatic conditions were grouped together under the cluster of that species. This indicates that genetic diversity correlates with geographical distribution of wide spread annual medic species in Iran. Results showed RAPD is applicable as a complementary tool in taxonomic identification of *Medicago* at both species and population levels.

P1167. Morphological and molecular variation in *Desmodium sumichrasti* (Fabaceae) a potentially useful species in ecological restoration.

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In many ecological restorations is essential to introduce many of the native species that have been extirpated through the degradation process and are not longer present on or near the site. For successful reintroduction use of the appropriate ecotypes and knowledge of genetic variation of the species is necessary. For temperate forests in Mexico and Central America there is no information regarding phenotypic and genetic variation of native shrub species. One such species is *Desmodium sumichrasti* (Fabaceae) a nitrogen fixing species with potential for restoration of temperate forests. Morphological variation in 80 herbarium specimens (70 characters were considered) and RAPD primer analysis of four populations was carried out. Results showed high variation among populations that increases with geographic distance. Due to the high morphological and genetic variation, for choosing collecting sites for ecological restoration, closest populations to the restoration site are recommended.

P1168. A phylogenetic analysis for 44 species of Old World *Trifolium* as well as 4 species from New World

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A phylogenetic analysis for 44 species of Old World *Trifolium* as well as 4 species from New World of all 8 sections and for two outgroup taxa (*Trigonella foenum graecum* and *T. stellata*) using 50 morphological characters were conducted. The results strongly supported monophyly of the genus. Species of section *lotoidea* are sisters to the remainder species of the genus. Sections *Vesicaria* and *Chronosemium* form monophyletic groups, separately, with high bootstrap value. The two largest cosmopolitan sections, *Lotoidea* and *Trifolium* are not, however, monophyletic. The New World species (*T. nanum*, *T. longipes*, *T. pinetorum* and *T. depauperatum*) are nested clearly within the old world ones and form a paraphyletic group in spite to previous treatments. The two species (*T. nanum*, *T. longipes*) of section *lotoidea* are basal within the genus that supporting the hypothesis of New World origin for the genus.

P1169. A Morphological Evaluation of *Medicago* Pollens

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Having more than 23 species of *Medicago*, Iran is one of the origins and centers of diversity of this genus. Because of close similarity in sexual organs and pod-characteristics, taxonomical classification of these species based on morphological characteristics is difficult. Scanning Electron Microscopy (SEM) was used to study the major characteristics of pollen among Iranian *Medicago* species. Pollens were mostly tricolpate, but quadricolpate or hexacolpate pollens were also seen in one species. The equatorial view was ovalish rectangular or spherical shapes and the polar view was obtuse-triangular, truncate-triangular or square tips. Majority of species showed reticulate (perforate, faveolate, regulate and scrobiculate) and verrucate exine surface. *M. rigiduloides* with quadric or hexic colpate pollen was barely distinguishable from *M. rigidula* with tricolpate pollen exine surface. On the other hand, *M. tornata* with its spherical pollen and verrucate exin surface was obviously different from *M. polymorpha* with rectangular pollen and reticulate exine surface. This is the first report of *M. rigiduloides* and *M. tornata* from Iran.

P1170. Cladistic and phenetic analysis of the relationships in *Vicia* subgenus *Cracca* (Fabaceae) based on morphological data

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The variation of 89 multistate morphological characters of 44 species from *Vicia* subgenus *Cracca* Peterm. was investigated with cladistic and phenetic methods using *Lathyrus sylvestris* L. and *Pisum elatius* M. B. as outgroups. The subgenus appears monophyletic, the *Ervum* group is supported as a section. The

sections *Vicilla* (Schur) Aschers. & Graebner and *Cassubicae* Radzhi appear monophyletic when *V. amoena* Fischer is moved to the section *Cassubicae*. Placement of *V. biennis* L. in a separate section is supported, while the sections *Variiegatae* Radzhi and *Panduratae* Kupicha are nested in the section *Cracca* Dumort and section *Pedunculatae* Rouy is not supported as monophyletic. Use of different outgroups results in slightly different substructures for sect. *Cracca*. Differences in the placement of some species (*V. onobrychioides* L., *V. megalotropis* Ledeb. and *V. biennis*) as well as in the structure of the subclades of the section *Cracca*, depending on the outgroup choice, clearly reveal the level of steadiness or instability of some groupings formed in the analysis of the morphological characters.

P1171. Molecular phylogeny of *Acacia* subgenus *Phyllodineae* (Leguminosae: Mimosoideae) based on nuclear DNA sequences

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Acacia subgenus *Phyllodineae* is a diverse assemblage including over 950 species, most of which occur in Australia. Chloroplast DNA evidence supports the monophyly of subg. *Phyllodineae*, with its probable sister taxon in the tribe Ingeae. Within *Phyllodineae* relationships of taxa have remained largely unresolved. In this study sequences of the ETS and ITS were used to test the monophyly of the seven currently defined sections. It is now clear that most of these sections are not monophyletic; instead the taxa are resolved into three main clades.

Previous studies assumed a natural division between taxa with uni-nerved phyllodes (section *Phyllodineae*) and those with pluri-nerved phyllodes (*Juliflorae* and *Plurinerves*). However, this division is not supported by sequence data of rDNA. Section *Phyllodineae* is polyphyletic, indicating that phyllode nerve-number is homoplasious. Also at least two separate reversals to the plesiomorphic state of bi-pinnate foliage have occurred. There is support for a modified version of Vassal's section *Pulchelloideae*, bringing together disparate taxa from sections *Alatae*, *Pulchellae*, *Phyllodineae* and *Lycopodiifoliae*.

P1172. Molecular Phylogeny of the Old World *Astragalus* L. (Fabaceae) as Inferred from nrDNA ITS Sequence Data

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Phylogenetic relationships among 230 species of the Old World *Astragalus* and 2 of the New World one were inferred from analyses of nuclear rDNA ITS sequences. The results of phylogenetic analyses suggest that all *Astragalus* species sampled, except taxonomic outliers including three annual species, *A. epiglottis*, *A. annularis* and *A. vogelii* (newly established as *Podlechiella vogelii*) and six perennials (from sect. *Phyllobium*), are united in a single clade, so-called *Astragalus* s. str. Our results show that *P. vogelii* is allied with an assemblage of *Colutea persica* and a clade of six species of sect. *Phyllobium* at the base of the trees. The three monotypic annual segregate genera *Thlaspidium* (= *A. thlaspi*), *Barnebyella* (= *A. migpo*), and *Ophiocarpus* (= *A. ophiocarpus*) are clearly nested within *Astragalus* s. str. Among sections analyzed, only sects. *Acanthophaea*, *Ammodendron*, *Bucerates* (including monotypic *Cyamodes*), *Cenanthrum*, *Caraganella*, *Chronopus*, *Eremophysa*, *Incani*, *Laxiflori* and *Lotidium* appear to be monophyletic.

Key words: *Astragalus*, Fabaceae, nrDNA ITS, Phylogeny

P1173. *Vicia cracca* and *V. tenuifolia* in Eastern Europe

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Vicia cracca and *V. tenuifolia* are two closely related species of Fabaceae widely distributed in Europe and Mid-Russian Valley. *V. cracca* usually inhabits forest and forest-steppe zones. *V. tenuifolia* is primarily a steppe species but it can be found much further north. Russian scientists traditionally delimit these species as in natural habitats they can be distinguished by general appearance. But the exact defining characters are uncertain. That's why *V. tenuifolia* is treated as a subspecies of *V. cracca* by many European authors (Hegi, 1924; Guinea, 1953; Davis, 1978). On

the other hand, overlapping habitats of these taxons require them to be regarded as species.

With the purpose of settling this question we've analyzed 10 defining characteristics of the vetches from Mid-Russian forest-steppe where habitat overlapping is great. These included length and width of the leaflets and their ratio, leaf and raceme lengths and ratio, leaf and stem pubescence, corolla and limb length and their ratio, raceme length and density. Raceme and flower characteristics define *V. tenuifolia* as a separate species. Further research on legume characteristics is being conducted.

P1174. Systematics of the Legume Fossils from the Miocene Shanwang Flora of Shandong Province, Eastern China

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The Miocene Shanwang flora from Shandong Province, eastern China has provided beautifully preserved legume fossils since the 1930's. To date, 9 legume genera have been reported, including *Albizia*, *Cercis*, *Gleditsia*, *Gymnocladus*, *Indigofera*, *Podogonium* (or *Podocarpium*), *Pueraria*, *Sophora*, and *Wisteria*. However, the systematics of many of these legume fossils are uncertain and in need of further study. Attention needs to be given to a detailed reinvestigation of their biological characters such as leaflet architecture and associated reproductive organs. Also, their phylogenetic and biogeographic significance needs to be assessed. These legume fossils are usually preserved in the diatomaceous shales, so their leaflet architecture and associated fruit characters are often exquisitely preserved in great detail so that careful comparisons can be made between the characters of the fossils and those of extant species. Based upon this potential to understand, the systematics and their biogeographic significance of these legume genera from the Shanwang flora should be reappraised in the future research.

P1175. Morphology, Diversity and Phylogeny of *Paloue*, *Elizabetha*, *Heterostemon* and Related Caesalpinoid Legumes from Northeastern South America

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Most phylogenetic analyses of species level relationships in the Leguminosae have focused on the subfamilies Mimosoideae and Papilionoideae and only recently have efforts been directed at groups within the subfamily Caesalpinioideae. Most phylogenetic work within the subfamily concentrates on higher level relationships among the tribes, subtribes, and genus groups. Over half of the genera of Caesalpinioideae are grouped in the large Detarieae s.l. clade. Within this clade, *Heterostemon* (7 spp.), *Elizabetha* (10 spp.), *Paloue* (4 spp.) and *Paloveopsis* (1 sp.) are endemic to northeastern South America and are thought to be closely related. This study uses morphological, anatomical and molecular characters to explore generic limits and relationships among these genera. Preliminary results support the monophyly of *Heterostemon*, *Elizabetha*, and *Paloue*, and also support the recognition of *Paloveopsis* as a distinct monospecific genus. Patterns of floral evolution implied in these results will be discussed in a phylogenetic context.

P1176. Pollen morphology in Iranian species of *Trifolium* sect. *vesicaria* using pollen morphology

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The species of *Vesicaria* section and *T. spumosum* of sect. *Mistyllus* collected from various locations in Iran, were used in this study. Pollen grain characteristics of species were evaluated by Scanning Electron Microscopy (S.E.M.). Characteristics of pollens such as polar length (P), equatorial length (E), P/E ratio, furrow length and ornamentation of exine surface were studied. The largest pollen belonged to *T. fragiferum* and smallest one to *T. clusii*. The pollen grains were oblong, trizoncolporate and triangular-obtuse in polar view and exine surface were regular or irregular reticulate. A cluster analysis divided the examined species into five groups. *T. fragiferum* and *T. clusii* were allocated in two different groups. *T. physodes* joined to *T. spumosum* of section *Mistyllus* in another group. *T. tumens* was separated in a different group and three annual species, *T. resupinatum*, *T. tomentosum*,

T. bullatum were classified in another group. The relatedness of *T. spumosum* to sect. *Vesicaria* is very high, which can be classified in the same section. A Factor Analysis indicated that characteristics were the most sources of variation compared to other grains. .

P1177. Phylogeny and taxonomy of the genus *Lotus* (Leguminosae): evidence from nrITS sequences and morphology

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Lotus is the largest genus of the tribe Loteae. It includes important crop plants and a model legume, *Lotus japonicus*. Taxonomy of *Lotus* is complicated, and a comprehensive taxonomic revision of the genus is needed. We have conducted phylogenetic analyses of *Lotus* based on nrITS data and morphological characters. We have included a lot of species not used in previous molecular (Allan et al. 2003, 2004) and morphological (Arambarri 2000) phylogenetic studies. Most important conclusions are: (1) previous findings that *Dorycnium* and *Tetragonolobus* cannot be separated from *Lotus* at the generic level are well supported; (2) *L. creticus* should be placed into section *Pedrosia* rather than into section *Lotea*; (3) wide treatment of section *Ononidium* is unnatural; (4) section *Lotus*, in its traditional circumscription, is not monophyletic; (5) *L. aegaeus* is closest to *L. gebelia*; (6) segregation of *L. shimperi* and allies into section *Chamaelotus* (Kramina & Sokoloff 2003) is well supported.

P1178. Evolution in the tribe *Hedysareae*

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5 genera of Iranian *Hedysareae* tribe (Fabaceae) have been studied. *Taverniera* (3 spp.), *Alhaji* (1 spp.), *Eversmannia* (1 spp.), *Hedysarum* (16 spp.), *Onobrychis* (64 spp.). The systematic position of these genera in *Hedysareae* was studied using testa micro- and macromorphology. Structure, distribution and taxonomic importance of foliar stomata in specimens of these taxa have been studied. In the specimens belong to the genus *Taverniera* (primitive genus) and *Eversmannia* (advanced genus), four stomatal types, anisocytic, staurocytic, brachyparacytic and anomotetracytic have been recognized. Apomorphic and plesiomorphic states are suggested for seed testa, phytodermological and morphological characters. Shrubby habit with long internodes simple leaves, short and few flower, reticulate sculpturing on seed coat, and dominance stomatal types anisocytic and staurocytic are the most important plesiomorphies, often observed in primitive genus tribe as *Taverniera*. Woodiness represents an advanced state in *Eversmannia*. Woodyfication along with simple leaves and reticulate pattern is a primitive state in tribe *Hedysareae*.

P1179. Taxonomy of *Heteropterys* subsection *Stenophyllarion* (Malpighiaceae) in Brazil

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Heteropterys is the second largest genus of Malpighiaceae, showing as autapomorphy the schizocarpic fruit, with each mericarp with a large dorsal wing with inferior margin thickened. *Heteropterys* comprises two subgenus, four sections and six subsections. Recent studies pointed out the monophyletism of subsect. *Stenophyllarion*. The species of this group occur as vines from Southeastern states of Brazil to Argentina, mostly in the Atlantic coastal forests, and are characterized by the petiole glandular at the base, the leaves glabrous or glabrate and the umbels with small short-peduncled flowers. Field work, anatomical studies and analysis of herbarium collections (including types) allow us to accept eight species: *H. crenulata*, *H. fluminensis*, *H. intermedia*, *H. leschenaultiana*, *H. pauciflora*, *H. microcarpa*, *H. wiedeaana* and one new species. Many new synonyms were proposed and lectotypes designated, specially when original collections were based on syntypes.

P1180. A systematic analysis of *Heteropterys* (Malpighiaceae) based on molecular data

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Heteropterys is the largest genus of Malpighiaceae with ca. 140 species. All species are found in the Neotropics except *H. leona*, which occurs in both the Caribbean and coastal West Africa. The genus is characterized by a noteworthy synapomorphy: the dorsal wing of the samara is elongated and thickened along the abaxial edge, and the lateral wings are strongly reduced or absent. A molecular phylogenetic analysis was performed on the genus using plastid (*matK*, *nadhF*, *rbcL*, *trnL-F*) and nuclear (*nrITS*, *PHYC*) sequence data. Independent and combined analyses show similar results: the genus is monophyletic, and subgenus *Parabanisteria*, subsections *Aptychia* and *Stenophyllarion* (subg. *Heteropterys*), and series *Metallophyllis* (subg. *Heteropterys*) all form well-supported clades. The series *Madarophyllis* are included with *Metallophyllis* clade and the subgenus *Heteropterys* and series *Xanthopetalis* not reveal a monophyletic group. However, future rearrangements will be necessary to improve the infrageneric taxonomy of the genus. This study provides comments on representative taxa, geographical distribution, habitat, and pollen morphology.

P1181. Phylogenetic relationships of Quinaceae (Malpighiales): insights from trnL-trnF sequence data

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The small neotropical family Quinaceae, together with Ochnaceae and Medusagynaceae, forms a well-supported clade within Malpighiales. Phylogenetic analyses using molecular data (*rbcL*, *ndhF*, 18S) have resulted in unclear relationships and/or delimitation of these three families, the reason why they recently were merged into Ochnaceae sensu lato. In order to provide further insights into their relationships, we analysed DNA sequences of the trnL-trnF region of the plastid genome in combination with morphology. Quinaceae were recovered as a well-supported monophyletic group with Medusagynaceae as the closest sister taxon. Both lineages form the sister group to Ochnaceae. Reconstruction of the breeding system modes showed that perfect flowers (*Froesia*) are the ancestral state in Quinaceae, while dioecy (*Lacunaria*) and androdioecy (*Quiina*, *Touroulia*) are derived.

P1182. Leaf morphology, anatomy and ultrastructure in four European Salix species

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Four European *Salix* species were analyzed: *S. alba*, *S. triandra*, *S. viminalis* and *S. purpurea*. The taxa belong to two subgenera and different sections. Preliminary observations showed a marked interspecific variation in leaf morphology, mainly concerning formation of the wax layer. A detailed analysis using SEM revealed that the layer varies from an amorphous film (*S. viminalis*) to large crystalloid structures (*S. alba*). Additional anatomical and ultrastructural investigations with LM and TEM provided valuable information, which supplements the scarce published data. Anatomical investigations involved traits like: leaf blade thickness, stomata density, epidermis and hypodermis thickness, number of cell layers in palisade and spongy mesophyll. Interspecific variation was proved also by ultrastructural analysis. The study revealed substantial differences between the four taxa, which could be important for taxonomic research on the genus *Salix*.

P1183. Biosystematics and chorology of the genus Nymphaea L. (Nymphaeaceae) in Sardinia (Italy).

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Nymphaea L. is a cosmopolitan genus composed of ca. 50 species divided into five subgenera. Among them the subgenus *Nymphaea* Planchon [=subgen. *Castalia* (Salisb.) DC.], characterized by diurnal anthesis, is present in the Mediterranean region, Sardinia and Sicily islands included, with one species *N. alba* L. Recently, the presence of this taxon in Sardinia and Sicily was given as "probably extinct" or "perhaps extint".

In this note the results of one-year-long observations on five natural populations, selected and sampled mainly in the N-NW Sardinia, are reported and the presence of *Nymphaea* in the island is obviously confirmed. Through the study of flower, fruit and seed morphology, rhizome and leaf morphology and anatomy and from first observations on phenology and reproductive biology, the attribution of the Sardinian "morphotypes" to the taxon *N. alba* is discussed. A citotaxonomic study, based on chromosome counts from germinating seeds is also presented.

P1184. Diversity of putative nymphaealean waterplants in the Lower Cretaceous of the Araripe Basin, Brazil

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The Aptian Crato Formation (Sergipea variverrucata palynozone) of NE Brazil contain angiosperm remains, mostly magnoliids. These include three taxa of water plants with nymphaealean features, such as creeping rhizomes and peltate leaves. The first taxon bears thin ovate petiolate peltate small leaves with actinodromous venation. Solitary flowering structures on peduncles also derive from the rhizome. The flowers contain up to 12 apocarpous free carpels, including small seeds with hilum, that are spirally attached to the flat receptacle. Carpel arrangement and seeds are similar to the Cabombaceae. The second taxon has petiolate, nearly orbicular, crenate, actinodromous leaves. Floral features are missing, but its general habit is close to that of members of extant Nymphaeaceae. The third taxon, also characterized by a horizontal rhizome with roots, bears relatively large petiolate peltate leaves with an entire to slightly sinuate margin and an actinodromous venation. One solitary multipartite flower sits on a thick peduncle of which the anatomical features are preserved. This plant might belong to the Nymphaeales or alternatively to the Nelumbonaceae.

P1185. White water-lily flowers behavior (Nymphaea candida J. et C. Presl.) in different photoperiods

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Water lily (*Nymphaea*, Nymphaeaceae) flowers are established as a good example of "plant clock". Our aim is to find the main factors that cause the behavior of *Nymphaea candida* flowers in natural habitat during different photoperiods.

Continuous observations were held during three field seasons on 41 flowers in Northern Russia population for six 24-hours days in Arctic day photoperiod; and 91 flowers in Middle Russia for 15 days in natural conditions, plus also in experiments without light and with single flash illumination in the mid-night. We estimated the degree of openness and degree of submergence of each flower.

Common tendencies for the change of flower openness look like circadian cycles. The degree of flower submergence changes with hardly seen periodicity, which usually expressed in weak nonlinear trend. Therewith, we observed coordinated changes of flower openness and flower submergence in one lake of Northern Russia and did not observe them in the other lake.

We propose that flower openness and flower submergence change independently of each other and their changes are synchronized by the changing of dark and light day-periods.

P1186. Host specialization in Orobanche foetida

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Orobanche foetida is widely distributed in Mediterranean area on wild legume plants, but has not been reported on cultivated legume species, except on faba bean in Tunisia.

A population of *O. foetida* collected on *Astragalus lusitanicus* in Spain and another population collected on faba bean in Tunisia were studied to compare the pathogenicity on faba bean. The Spanish population was no infective on faba bean and seeds were poorly stimulated by all faba bean accessions. However, a substantial proportion of geminated seeds were able to contact and start attachment of faba bean roots, although failed to get

established due to a frequent hypersensitive like host root cells reaction. On the contrary, the Tunisian population was very infective on faba bean and seeds germinated profusely in all faba bean accessions, with a higher success in attachment to faba bean roots and almost null hypersensitive like reaction, resulting in a high establishment. Some genotypic variation in resistance was observed in faba bean germplasm. Resistance against *O. crenata* was not operative against *O. foetida*, with the exception of cv. Baraca, the most resistant to both species.

P1187. Evolution of phytochromes in parasitic *Orobanchaceae*

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The evolution of heterotrophy in plants may involve the evolution of new morphological features such as haustoria, but there may also be reduced selective pressure for the maintenance of features associated with photosynthesis. Some developmental processes mediated by phytochrome photoreceptors, such as leaf and chloroplast development and the expression of photosynthetic genes are altered in parasitic plants. *Orobanchaceae* contains both holoparasitic and hemiparasitic species and so offers a system in which to investigate how the functions of phytochromes might be altered in parasitic plants. We have used DNA sequences of *PHYA*, the gene encoding phytochrome A, to infer a phylogeny for the family. The well-supported gene phylogeny suggests that at least some hemiparasites and holoparasites have two copies of *PHYA* and that some *PHYA* genes harbor novel introns. There is also evidence that *PHYA* sequences are evolving under relaxed constraints and we are investigating patterns of molecular evolution in more detail to determine whether selective pressures change along branches to major clades or whether they might be closely correlated with shifts in habit.

P1188. Genetic diversity in two variants of *Orobanche gracilis* Sm. [var. *gracilis* and var. *deludens* (Beck) A. Pujadas] from different regions of Spain

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The pattern of genetic variation among populations of two taxa of *Orobanche gracilis* Sm. [var. *gracilis* and var. *deludens* (Beck) A. Pujadas] from Northern and Southern Spain growing on different hosts was analysed using RAPD markers. The Dice's distance matrix was analyzed by UPGMA method and the resultant dendrogram clearly divided populations by region and botanical variety with the Southern populations being more differentiated among them in both cases (var. *gracilis* and var. *deludens*). Concerning *O. gracilis* var. *deludens*, the dendrogram has also shown host differentiation between populations growing on two different species, *Ulex baeticus* and *Ulex parviflorus*. A complementary analysis of principal coordinates (PCoA) confirmed these results. The analysis of molecular variance (AMOVA) indicated that the lowest level of differentiation was found among the Northern *O. gracilis* var. *gracilis* populations whereas in the case of *O. gracilis* var. *deludens* from the South most of the genetic diversity was attributable to differences among populations.

P1189. Hemiparasitism: a way station to holoparasitism or an evolutionary stable strategy?

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Is hemiparasitism an evolutionary stable strategy (ESS) or just a way station on the road from autotrophy to holoparasitism? Studies on the obligate hemiparasite *Pedicularis canadensis* (Rhinanthaceae) suggest that in prairie communities where nutrients or water are very limiting factors, parasitism is an adaptation both for enhancing acquisition of nutrients, particularly nitrogen, and/or water and for enhancing photosynthesis, thus maintaining both systems. The presence of the hemiparasite even enhances species diversity by extracting a higher cost upon dominant species, like grasses, in comparison to ephemerals like shooting star, *Dodecatheon meadia*. This lousewort species also inhabits forest communities in eastern North America where the prairie model predicts increased gain via parasitism without

enhancing photosynthesis. If so, the lousewort's photosynthetic potential should decline in forest communities shifting the balance toward holoparasitism.

P1190. Anatomical features of the stem structure in the *Orobanchaceae*

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All the of *Orobanchaceae* are parasitic plants without chlorophyll. This family consists of nearly 225 species belonging to 16 genera. The anatomical structure of the stem in 87 species of 13 genera has been studied.

In connection with parasitic way of life this plants have very simple morphological structure. Their anatomical structure become more develop, especially their excretory system. There are two excretory mechanisms (by the glandular hairs in *Orobanche*, and by the epidermal cells in *Cistanche*) were found.

As the anatomical data on stem structure shown, the *Orobanchaceae* is a natural taxon that should not be regarded as subfamily *Orobanchoidae* of the family *Scrophulariaceae*. The taxonomic position of the genus *Lathraea* in *Scrophulariaceae* is confirmed, however, by its stem anatomy. The diagnostic value of some stem features of *Orobanchaceae* has been established. The sections of genera *Orobanche* and *Cistanche* are well distinguished by features of stem anatomical structure.

P1191. The Figwort Family (Scrophulariaceae Juss.) in Belarussian Flora

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Scrophulariaceae is one of the largest family of Angiosperm in the temperate areas of the North hemisphere. The objects of our investigation include aboriginal and synantropical components of Scrophulariaceae (incl. *Orobanchaceae* Vent.) in Belarus. The work comprises critical taxonomic revision of complex groups, nomenclatural, Caryological, ecological and phytocoenological information about all species of Figwort family in this region, with an issue of 8 years work of the author, with field experiences and revision of more than 7 000 specimens of the herbaria KRAM, KW, LE, LECB, LW, LWS, MHA, MSK, MSKU, MW, WIR etc. The phytogeography of the Scrophulariaceae in this region is compared to the flora in other neighbour areas (Poland, Baltic countries, adjacent regions of the Ukraine and Russia). Among the main contributions in our work are also presented an analytical key for the identification of the taxa, dot maps of the distribution of the endangered, rare and critical species, as well as information about protection and sustainable use of representatives of the Figwort family in Belarus.

P1192. Phenetic analyses of morphological traits in the *Alectra sessiliflora* complex (*Orobanchaceae*)

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Alectra (*Orobanchaceae*) is a genus of primarily hemiparasitic herbs. *Alectra sessiliflora* var. *sessiliflora*, var. *senegalensis* and var. *monticola* form a morphologically cohesive group with overlapping distributions throughout sub-Saharan Africa, and are characterized by sessile yellow flowers and a branching habit. Traditional characters used to distinguish varieties were stamen filament pubescence or calyx pubescence. These characters are degenerate, preventing confident placement of a single specimen within one variety. This difficulty suggests that the taxonomic delimitations may not be meaningful. A phenetic study was undertaken to determine if this complex is best treated as a single large polymorphic taxon, or as a series of similar yet discrete taxa. Seventeen morphological characters were examined on 134 operational taxonomic units (OTUs), and the data were analyzed using UPGMA, PCA and discriminant analyses. Discrete clusters were not identified by the analyses.

P1193. Distribution patterns of the genus *Piper* (*Piperaceae*) in a South-Ecuadorian montane rain forest

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The studies were conducted in the area of the Estacion Cientifica San Francisco which is situated on the eastern slope of the

Cordillera El Consuelo in Southern Ecuador. The data were obtained from 15 permanent plots in ravines and ridges along an altitudinal gradient between 1850 and 2450 masl. All (morpho-) species of the genus *Piper* were registered with height and diameter. 701 individuals belonging to 21 species were counted and identified. Data on abiotic factors obtained by former investigations were used to explain observed distribution patterns. The results indicate no significant impact by the altitudinal gradient on the number of species per plot. There are significant differences in species composition and distribution between ridge and ravine forests. The overall species richness is higher in the ravine forests plots. About half of the species identified are exclusively abundant in the ravines whereas the other half are common in both, ravines and ridges. Ridge forests comprise only a small amount of unique species. The average number of individuals and species of *Piper* per plot are significantly larger in the ravine forests.

P1194. Heterotopic expression of petal identity gene homologs in petaloid-bracts of *Houttuynia cordata*.

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Heterotopy has been thought to be involved in the evolution of some novel structures in plants as well as animals. Petaloid-bracts are often associated with the evolution of pseudanthia and are reported from many angiosperm taxa. However, genetic mechanisms underlying their evolution are largely unknown. Recent molecular genetics studies showed that MADS-box genes play a key role in determining floral organ identity in flowering plants. One of the possible mechanisms responsible for the evolution of petaloid-bracts is heterotopic expression of petal identity genes in their development. To test this hypothesis, we cloned MADS-box genes from *Houttuynia cordata* (Saururaceae), which forms pseudanthia with white petaloid-bracts. Expression analyses of these genes by RT-PCR and *in situ* hybridization revealed that putative orthologs of the genes necessary and sufficient to determine petal identity in *Arabidopsis* are expressed in petaloid-bracts of *H. cordata*. Based on these results, we will discuss genetic control of petaloid-bract development and its evolution in *H. cordata*.

P1195. Phylogeny of the western Mediterranean species of the genus *Aristolochia* (Aristolochiaceae)

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Molecular results inferred the Mediterranean species as the most derived Aristolochiaceae, being part of an extended genus *Aristolochia*. An Asian origin of the paleotropical and mediterranean representatives has been considered in previous analyses. So far, the relationships and taxonomy within this clade are not yet specified and are discussed controversially. Current understanding of relationships is based only on morphological characters.

A phylogeny, based on coding and non-coding chloroplast sequences of the Mediterranean *Aristolochia* species is presented with insights into evolution, radiation and phylogeography. All clades gain maximum support. *Aristolochia* in the Mediterranean in total is monophyletic with the two Mediterranean lianas forming a monophyletic clade as well. Relationships previously proposed within the tuber-forming taxa, could not be confirmed. The multiple independent evolution of the geophytes is interpreted as an adaptation to different habitats.

P1196. Molecular phylogeny and revision of *Aristolochia sensu lato* (Aristolochiaceae), as inferred from *rbcl*, *matK*, and *PHYA* genes

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Taxonomic treatments of the genus *Aristolochia sensu lato* (400 spp.) have been ambiguous and controversial, especially on the generic status. In a recent cladistic analysis by morphological characters, it was proposed that it should be divided into four genera of two subtribes. To elucidate the phylogenetic

relationships within the genus *Aristolochia sensu lato*, this study reconstructed the phylogeny on the basis of nucleotide sequences in chloroplast *rbcl* gene and the nuclear encoded phytochrome A (*PHYA*) gene for representative species, and those of *matK* gene for ca. 90 spp. All phylogenetic trees by three genes indicated that *Aristolochia sensu lato* consisted of two major lineages. The only *matK* phylogeny showed that each of two lineages included two sublineages, respectively. The chromosome numbers were also congruent the phylogeny. Considering the phylogeny, morphology, and chromosome numbers, we propose a revised system that classified *Aristolochia sensu lato* into two genera and four subgenera; *Aristolochia* subgenera *Aristolochia* and *Pararistolochia*, and *Isotrema* subgenera *Isotrema* and *Endodeca*.

P1197. Chloroplast DNA variation and geographic structure of the *Aristolochia kaempferi* group

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The present study documents cpDNA variation in the *Aristolochia kaempferi* group (Aristolochiaceae), which consists of one Chinese and all Japanese and Taiwanese species of subgenus *Siphisia*, i.e. *A. kaempferi*, *A. liukiensis*, *A. shimadai*, *A. cucurbitifolia*, and *A. mollissima*. Phylogenetic analysis based on the nucleotide sequences of *matK* gene, *atpB-rbcL* and *trnS-trnG* intergenic spacers of 138 individuals from 95 populations over their entire distribution range in Japan and Taiwan were carried out. A total of 38 haplotypes recognized in the analysis formed distinct six clades, that were not consistent with previous taxonomic classifications. However, the clades had significant association with geographic distribution of haplotypes, that was tested by the nested clade analysis (NCA). Phylogeographic processes inferred by NCA were consistent with hypothesis of range expansion from some refugia after the last glacial maximum. The cpDNA phylogenies being better correlated with geographical distribution than with taxonomic boundaries in the *A. kaempferi* group may suggest the possibility of hybridization and introgression, and / or parallel evolution.

P1198. Phylogeny and biogeography of *Piper* sect. *Macrostachys* (Piperaceae)

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Phylogenetic and biogeographic patterns were examined in the Neotropical *Piper* sect. *Macrostachys* (Piperaceae) using ITS sequence data. *Piper* sect. *Macrostachys* is composed of 35-50 species with centers of diversity in Ecuador and southern Colombia, and southern Central America. Low levels of molecular divergence suggest that this section has undergone rapid and recent diversification and this hypothesis was tested with a molecular clock. Although analyses produced only partially resolved trees, our results strongly support a monophyletic sect. *Macrostachys* and several well supported internal clades that largely correspond to morphological groupings and geographic areas. Because of the low resolution in the phylogeny and the finding that the basal most species of the section are widespread, the geographic origin of the section remains unclear. Our results do suggest, however, that diversification in *Piper* sect. *Macrostachys* did not occur over a long time in a stable tropical climate, but rather was linked with geologic and climatologic events associated with the uplift of the Andes and the Isthmus of Panama over the past 2-5 million years.

P1199. The Phylogeny and Taxonomy of Magnoliaceae

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The family Magnoliaceae is one of the most primitive groups of angiosperms and has played an important role in the research of the origin, evolution and systematics of flower plants. In this paper author has reviewed the history and new progress of taxonomic and phylogenetic research, especially the controversial views and insufficiencies of current systems such as Y. H. Law's (1998) and R. B. Figlar & H. P. Nooteboom's (2004). Some important species with special taxonomic meanings are discussed and some new taxa are described. Moreover the new phylogenetic viewpoints, new cladogram and new system of the family are put forward. The

system includes 3 subfamilies: Michelioideae (Law) D. L. Fu, subfam. comb. nov., *Magnolioideae* and *Liriodendroideae* (Bark.) Law, 5 tribes: *Michelieae* Law, *Yulanieae* D. L. Fu, trib. nov., *Alcimandrieae* (Law) D. L. Fu, trib. comb. nov., *Magnolieae* and *Kmerieae* D. L. Fu, trib. nov., 10 genera: *Michelia* L., *Paramichelia* Hu, *Yulania* Spach, *Alcimandra* Dandy, *Gynopodium* (Dandy) D. L. Fu, gen. comb. nov., *Magnolia* L., *Talauma* Juss., *Pachylarnax* Dandy, *Kmeria* (Pierre) Dandy and *Liriodendron* L..

P1200. Evolutionary patterns in Afro-Malagasy *Monodora* and *Isolona* (Annonaceae)

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A systematic and anatomical study of the genera *Monodora* (c. 14 spp.) and *Isolona* (c. 19 spp.) is being undertaken as part of a revision of all African Annonaceae. Both genera have a tropical African distribution, with *Isolona* also present in Madagascar. They have paracarpic gynoecia, which are unique within the family, but they differ in the number and arrangement of the carpels. A molecular phylogeny of Annonaceae using plastid DNA sequences confirmed the monophyly of these two genera. However, *Monodora* and *Isolona* are distinct in their perianth morphology (weak gamopetal, foliaceous and differentiated petals in two whorls vs. advanced gamopetal, thick and equal sized petals in one whorl) and palynology (tetrads vs. monads) suggesting divergent pollination syndromes. A taxonomic revision is underway with a few newly identified species particularly from Tanzania. Generation of a phylogeny of both genera and their putative sister taxa using plastid and nuclear genes as well as morpho-anatomical characters, will be used to determine evolutionary patterns and to reconstruct the biogeographical history in the context of other tropical African taxa.

P1201. Factors limiting fruit production in *Aristolochia paucinervis* Pomel

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Factors determining the fruit set of *Aristolochia paucinervis* Pomel (Aristolochiaceae) were studied in two different populations located in SW Spain during two consecutive years. This species is a Mediterranean tuberous perennial herb with protogynous trap flowers. To perform this study, flower production, fruit initiation, fruit abortion, fruit predation and fruit production were quantified. *Aristolochia paucinervis* showed a low fruit set in both populations during these two years. Low fruit initiation was the main factor limiting fruit production. Analysis of pollen tubes in stigmas suggested that the low fruit initiation was a consequence of deficient pollination. On the other hand, about 50% of the flowering plants did not initiate any fruit. The correlations found between the tuber weight and the number of flowers and fruits could indicate a gender diphasy situation, i. e. smaller plants only contribute to male function as pollen donors while bigger plants are hermaphrodites. Thus, gender diphasy and deficient pollination explain the low fruit set found in *Aristolochia paucinervis*.

P1202. Contributions to karyosystematic of *Veronica* L. (Scrophulariaceae)

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The genus *Veronica* is characterized by a great karyological variability. Only in the annual taxa, more of six basic numbers have been found: 6, 7, 8, 9, 13 and 15. Aneuploidy and polyploidy together with other somatic processes have originated a lot of chromosome numbers and several ploidy levels.

This work is focused on the karyosystematic of some of the annual *Veronica* species present at the Iberian Peninsula and belonging to subgenus *Cochlidiosperma* (*V. panormitana*, *V. trichadena*, *V. cymbalaria*, *V. sibthorpioides*, *V. triloba*, *V. hederifolia*), *Pellidiosperma* (*V. praecox*, *V. triphyllus*), *Triangulicapsula* (*V. chamaepithyoides*), *Pocilla* (*V. polita*, *V. agrestis*, *V. persica*, *V. filiformis*) and *Chamaedrys* (*V. verna*, *V. dillenii*). Some extra-iberical taxa have also been considered in order to check the validity of some hypothesis about phylogeny and some correlations between ploidy level and other taxonomical characters, such pollen size or seed coat-type.

P1203. Diversity of seed funicles in Scrophulariaceae s.l. and related families of the Lamiales

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The delimitation and taxonomy of Scrophulariaceae is still under debate. Among morphological characters, seed morphology is of great importance. However, little attention has been paid to the fact how the seeds are attached to the placenta and if the seed funicle provides systematically valueable characters. 26 genera/39 spp. of Scrophulariaceae s.l. were investigated by means of SEM. The study enabled recognition of 6 types, which partly correlate well with subgroups of Scrophulariaceae. The *Digitalis*-type, for example, is characteristic of the tribes Verbasceae, Digitalieae and Veroniceae (sensu Fischer, 2004); the *Manulea*-type is concentrated in tribes Alonsoeae, Aptosimeae and Manuleae, all centred in South Africa. Study of development and abortive ovules showed that the seed funicle is rather part of the placenta than part of the ovule. Random tests in other families of Lamiales showed that there are no seed funicles comparable to those of Scrophulariaceae.

P1204. Pollen morphology of some species of the the genus *Veronica* in Iran

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Iran is an important centre of diversity of this genus, with 56 species, among them 18 endemics (Saeidi, 2000). Pollen morphology of 17 species as representatives of five sections within *Veronica* distributed in Iran were examined by LM and SEM. Detailed pollen morphological characteristics are given for these species. Of these, 6 were not studied so far. Pollen samples were acetolyzed. Length of equatorial and polar axis were measured with aid of a x100 eyepiece. Measurement of grains was based on 30 grains per sample. Pollen grains are shed as monads. They are tricolpate (with three, equidistant, furrow-like apertures that are perpendicular to the equator which are meridional), or tetracolpate, isopolar and boat-shaped-oblong to spherical in equatorial view. They are also euprolate, radio-symmetric. Exine is tectate-columellate. The type of rugulate-perforate is more frequent between species examined and occurs in 14 species. Types of microreticulate and scabrate also observed in *V. beccabunga* and *V. hederifolia*, respectively. Our results confirm the eurypalynous character of the genus suggested by Hong (1984) and Fernandez et al. (1997).

P1205. Anatomical studies in relation taxonomy of Persian *Plantago* species

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Abstract: The present survey was performed on sixteen Persian *Plantago* species, and some of structural differences between the sections of the 16 species were evident. Thus we could see the contrast between the structural stem. This structures are follows: circular structure with scattered hairs in Sect. *Coronopus*, stellate structure in Sect. *Arnoglossum*, circular and undulate in Sect. *Plantago* and *AA*.

Stems have a range from no hair to a very hairy state, also in species which have hairy stems a ligneous cell is visible in the base of hair.

There is a layer of sclerenchyma fibres after the different layers of the cortex. This layer is placed similar to stem plan. The primitive species that is belong to *Psyllium* subgenus have more wooden parts than the advanced species that is belong to *Plantago* subgenus. In comparison between two subgenus, the species *Psyllium* subgenus have a thicker fibrous layer than the species *Plantago* subgenus. It seems as if the vascular bundles are inside this fibrous layer.

P1207. Study of micromorphology *Linaria* Sec. *Linaria* (Scrophulariaceae) in Iran.

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The genus of *Linaria* (Scrophulariaceae) contain 150 species in the world. From these species 32 occur in Iran. The genus has

been divided in 7 section on the base of shape and ornamentation of seed. Sec. *Linaria* have disc form winged seed. This section are variation species that almost distribution in extent of Iran. this section contain 50 species in world that 20 species occur in Iran. In this investigation to use of shape and ornamentation of testa cell seed and capsule, pollen, anatomy of stem and leaf that determined 5 new species from Sec. *Linaria* are for flora of the world. this investigation on morphological, pollenology, testa cell ornamentation, shape and surface seed and capsule to used SEM and anatomy stem and leaf. The specimens were collected from different regions in Iran and after recognition. Were studied for Numerical taxonomic studies, 70 qualitative and quantitative characters of morphological, pollen, seed, capsule and anatomy were selected for each specimen and finally species.

P1208. Wulfeniaceae Lakusic - New Family in Order Scrophulariales

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There is new family described in the order *Scrophulariales*. It is named *Wulfeniaceae* and it includes following genres: *Wulfenia* Jacq., *Wulfeniella* Lakusic, *Synthyris* Benth. and *Lagotis* Gaertn.

Genus *Wulfenia* Jacq. includes 5 species: *W. carinthiaca* Jacq. Carinthiacs Alps, *W. bleicii* Lakusic on North-East limestone Prokletije mountains, *W. rohlena* Lakusic on vulkanic Prokletije mountains, *W. orientalis* Boiss. on East Turkey and West Syria mountains, and *W. amherstiana* Benth. on Himalaya mountains. All species have

$2n = 18$.

Genus *Wulfeniella* Lakusic includes 2 species with $2n = 16$: *W. himalayca* (Hook) Lakusic on Himalaya mountains and *W. baldaccii* (Deg.) Lakusic on South-West Prokletija mountains.

Genus *Synthyris* Benth. with $2n = 24$, lives on N. Amerika mountains and includes 5 species: *S. missurica* (Bafin) Penn., *S. pinnatifida* Wats., *S. platycarpa* Gail & Penn., *S. reniformis* (Doug.) Benth. and *S. stellata* Penn.

Genus *Lagotis* Gaertn, which lives in Asia & N. Amerika mountains has 6 species: *L. uralensis* Schischkin, *L. cachmirian*, *L. glauca* Gaertn., *L. minor* (Willd.) Standl. & *L. tacedana* Miyabe & Tawaki, with $2n = 22$ & *L. missurica* (Rafin) Penn. with $2n = 44$.

P1209. Taxonomic problems within genera *Callitriche* in Poland

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The genera *Callitriche* (water starwort) cover six species in the flora of Poland: *C. hermaphrodita*, *C. hamulata*, *C. polymorpha*, *C. palustris* (= *C. verna*), *C. stagnalis*, and *C. platycarpa*. These plants are considered to be stenobionts in relation to different environmental factors, including the level of phosphorous and are used as bioindicators of water eutrophication. An accurate identification of species within *Callitriche* genera is difficult without developed fruits.

Preliminary studies of genetic variation of *Callitriche* species were carried out in Poland on the basis of enzymatic protein analysis. The analyzed systems were GOT, ADH and MDH. The number of chromosomes was determined in each sample. Two groups of plants have been separated: with $2n = 10$, (*C. polymorpha* and *C. stagnalis* - the both popular in Poland and difficult to differentiate), and a group with $2n = 20$ (*C. platycarpa* and *C. palustris*).

Large morphological variation within the species results from a significant plasticity of individuals adaptable to changing conditions of the environment or from their genetic variation.

P1210. The origin and development of floral shoot and pre-anthesis cleistogamy in *Hydrobryopsis sessilis* (Podostemaceae)

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Hydrobryopsis sessilis is a highly reduced, crustose and endemic member of subfamily Podostemoideae that grows attached to rocks in river waters. The dorsiventral plant body (interpreted as a stem) bears floral dwarf shoots all along the dorsal margin. This poster describes the reproductive biology of *Hydrobryopsis sessilis* with special reference to ontogeny of dwarf floral shoots, spatio-

temporal relationship of the floral parts, pollination and breeding system. Tracheids differentiate with the onset of flowering. Each floral shoot meristem is deep-seated, tiny, concave, and arises endogenously as a result of lysigeny. The *ab initio* dorsiventrality of the carpels has been seen. A novel feature of the ovules is the occurrence of endothelium. *H. sessilis* is unique as it shows pre-anthesis, complete, constitutional cleistogamy under submerged conditions. A significant finding is the presence of several pollen tubes in the filaments of stamens indicating a trend towards cryptic self-fertilization.

P1211. Studies in Mexican Podostemaceae: physiology in *Marathrum*

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The study was carried out in the Horcones and Las Juntas Rivers at south of Puerto Vallarta, Mexico. Field studies of leaves photosynthetic rate ($\text{ppm CO}_2 \text{ cm}^{-2} \text{ min}^{-1}$), leaves chlorophylls a (Chl a), b (Chl b) and carotenoids concentration (mg mL^{-1}), foliar area (cm^2) and biomass in plants of *Marathrum rubrum* and *M. schiedeanum* were conducted during March, June, September and December 2004. In reproductive phase photosynthetic rate was about 0.04051 (*M. rubrum*) and 0.6239 (*M. schiedeanum*); in vegetative phase values was about 1.0466 (*M. rubrum*) and 16.1183 (*M. schiedeanum*). In *M. rubrum* Chl a varied from 5.3092 to 8.4519, Chl b from 9.2631 to 38.1022 from reproductive to vegetative phase; in *M. schiedeanum* Chl a varied from 1.579 to 2.9193, Chl b from 1.0394 to 5.6195 from reproductive to vegetative phase; carotenoids show a similar behaviour with lower values than chlorophylls. Foliar area was greater in *M. schiedeanum* (from 134 to 343 cm^2) than *M. rubrum* (about 30% less); in reproductive phase some plants were observed with leaves in both species, more degraded in *M. rubrum*; this species had generally 20-25% less biomass than *M. schiedeanum*.

P1212. Studies in Mexican Podostemaceae: Morphological and embryological correlation in *Marathrum rubrum*

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Species of the Podostemaceae family inhabit tropical river-rapids and waterfalls; these plants grow firmly attached to the rocks. For their appearance, these organisms have been associated with mosses, briophytes and algae. Podostemaceae constitutes the largest family of strictly aquatic plants. *Marathrum rubrum* flowers were collected in the Horcones River, Jalisco, Mexico during flowering time (dry season) in 2003 and 2004. This work intends to correlate flower's external morphology, flower's position on the rock and the developmental stage of gynoecium and androecium. *M. rubrum* flowers were classified in five stages, according to the morphological characters they exhibit. The stages proposed were studied and characterized; the first stage begins when flowers still are in the spathe and the last one is when anthers have been lost and the pedicel acquires a brownish color. Ovules and anthers are fully developed since the first stage. Flower positioning on the rock is variable and is not associated with the developmental stage. Correlation exists between the flower length, external morphology and changes within the anthers.

P1213. Studies in Mexican Podostemaceae: Crossed pollination between two species of *Marathrum*

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Podostemaceae (river weeds) is the largest family among aquatic angiosperms. Studies concerning reproductive systems within the family are scarce. Current studies for *Marathrum rubrum* Novelo & Philbrick and *M. schiedeanum* (Cham.) Tul. demonstrate the close relation between both species. The study was carried out in the Horcones and Las Juntas rivers, Jalisco, México. Outcrossing studies were conducted between *M. rubrum* and *M. schiedeanum* in order to establish the degree of incompatibility. Flowers of *M. rubrum* were pollinated with pollen from *M. schiedeanum* and vice versa, prior emasculation and bagging to prevent natural pollination. The presence of pollen tubes in the stigma and placenta was confirmed by observation of flower samples stained with aniline blue in both species. Experimental evidence indicates

that incompatibility systems are absent in these particular outcrossings at least before the pollen tube comes in contact with the ovule.

P1214. Developmental anatomy of shoot in aquatic angiosperm *Weddellina squamulosa* (Podostemaceae)

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Podostemaceae are a family of aquatic angiosperms growing on rock surfaces in rapids and waterfalls and comprise three subfamilies which about 270 species and about 47 genera. In subfamily Podostemoideae examined the shoot has no apical meristem and new leaves form successively at base of developing young leaves. In Tristichoideae the shoot has an apical meristem and the leaves are formed on its flank. Here we show the unique pattern of shoot development of *Weddellina squamulosa*, the sole species of subfamily Weddellinoideae that is sister to Podostemoideae. The shoot is sympodially branched with branches oriented in a single plane. The shoot has an apical meristem of a tunica-corporis configuration, although it is eventually determinate. A new branch is formed near the base of a developing young shoot but not in the axil. Leaves are formed acropetally and tightly close to each other. Additional leaves are formed basipetally between developed leaves, while the leaves become separated by intercalary growth. The pattern of shoot branching and that of leaf development of *W. squamulosa* is compared with those of subfamilies Tristichoideae and Podostemoideae.

P1215. Molecular Phylogeny of Cameroonian and Ghanaian Podostemaceae

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All members of the Podostemaceae grow on rock surfaces in rivers, rapids, and waterfalls with swift running water. To survive in these extreme aquatic habitats, they have evolved unusual morphologies, such as flattened photosynthetic roots. The largest subfamily Podostemoideae has a pantropic distribution; previous molecular phylogenetic studies have analyzed a number of American, Asian species but only two from Africa (Madagascar) where molecular data is scanty. In this study, we examined the molecular phylogenetic relationship of about 20 Cameroonian and one Ghanaian species of the Podostemoideae. In the phylogenetic tree for the chloroplast *matK* gene, all our analyzed African and Madagascan species form a monophyletic group that is a sister clade to the Asian species. Our African species show morphological variation in the flower spathe (inverse vs. erect), plant size (acaulescent to having long shoot), and root (axial, ribbon-like or foliose). The morphological evolution of the African clade is discussed based on this phylogenetic analysis.

P1216. Application of Gliadin Markers to Estimate Biodiversity of *Triticum persicum* and *Triticum pyramidale*.

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We studied the genetic diversity of tetraploid wheats by examining seed storage protein markers at the Gli-A1 and Gli-A2 loci. High rates of electrophoretic polymorphism were detected at the loci studied in two selected species. In *T. persicum* a total of 72 allelic variants at 2 loci were detected, including 41 for Gli-A1 and 31 for Gli-A2. In the *T. pyramidale* samples, 48 allelic variant were observed. The numbers of alleles were 28 and 20 for Gli-A1 and Gli-A2 respectively. A null allele was detected at the Gli-A1 locus in 1 sample of *T. pyramidale* from Iran. At the Gli-A2 locus of 1 genotype of *T. pyramidale* from Turkey another null allele also was found. No null alleles were observed in the *T. persicum* samples. This observation confirms that in some samples these loci are inactive or semi-active and then some subunits are lacked. Some Iranian populations of *T. persicum* showed high rates of variation, but this variability was not observed in the *T. pyramidale* samples.

P1217. Low variability of internal transcribed spacer rDNA and *trnL* (UAA) intron sequences of several taxa in the *Festuca ovina* aggregate (Poaceae)

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Identification and classification of numerous *Festuca* species is still a difficult problem due to the close morphological resemblance. The most difficult fine fescues to identify belong to the *Festuca ovina* aggregate, which is the largest group in the genus *Festuca*. In order to evaluate the taxonomic value of DNA-based markers, sequence analysis of the internal transcribed spacer (ITS1-5.8S-ITS2) region and the chloroplast *trnL* (UAA) intron was performed in the ten most problematic fine fescues belonging to the *Festuca ovina* aggregate. Studied species from *F. ovina* group were: *F. pallens*, *F. vaginata*; species from the *F. valesiaca* group were: *F. dalmatica*, *F. pseudodalmatica*, *F. pseudovina*, *F. rupicola*, *F. valesiaca*; and species from the transitional group were: *F. javorkae*, *F. stricta*, *F. wagneri*. Intraspecific ITS variants were found in a single case while in other cases only intragenomic ITS polymorphisms were detected with 1-2 ambiguous positions. Among the sequences of the *trnL* (UAA) intron even intragenomic polymorphisms were not detected in any of the *Festuca* species studied. Thus, results do not appear to support the species status of these ten taxa.

P1218. A close phylogenetic relationship detected between *Poa* subg. *Andinae* of Patagonia and *Hookerochloa* and *Festucella* of Australia (Poaceae, Pooideae, Poeae): evidence from nuclear and chloroplast DNA sequences and morphology

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Phylogenetic analysis of ITS and trnT-trnF sequences of *Poa* and allies revealed a southern hemisphere relationship between *P.* subg. *Andinae* (coastal and interior wetlands, Patagonia) and *Hookerochloa* and *Festucella* (both of mesic highlands, Australia). The latter two monotypic genera were recently transferred to the Australian-New Zealand genus *Austrofestuca*, all 3 spp. having similar multiflowered spikelets, glabrous ovaries, oval hila, and bearded calluses. However, while both nuclear and plastid data resolve *Austrofestuca* s.s. within *Poa*, ITS data shows a well supported clade of *P.* subg. *Andinae*, *Hookerochloa*, *Festucella*, and *Arctagrostis* (arctic) outside of *Poa* among elements of Alopecurinae, Poinae, and Puccinelliinae. In our trnTF analysis these four taxa plus east Asian montane genus *Aniselytron* (ITS unknown) form a paraphyletic assemblage with similar sequences in a Poinae-Alopecurinae clade excluding *Poa*. Morphological data also support a close relationship among *P.* subg. *Andinae*, *Hookerochloa*, and *Festucella*, but a connection with the 1-flowered spikelet genera *Aniselytron* and *Arctagrostis* is not obvious.

P1219. Molecular phylogenetic investigations in the grass subtribe Poeae: Poinae and its relatives

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First results on matK-based phylogenetic estimations in grasses belonging to the subfamily Pooideae tribe Poeae and its presumed neighbor tribes as outgroups are presented. Since (i) the highly diversified generic assemblage around *Festuca* (Poeae subtribe Lolliinae) has recently been covered by molecular systematic studies of a dense taxonomic sampling and (ii) a comparable study in the tribe Aveneae has already been in progress, it became evident that similar approaches would be required also in further tribes and subtribes of the subfamily Pooideae. Our study aims at testing the "evolutionary lineages" suggested by the authors of different taxonomic concepts in Poeae and at including

preferentially those genera related with *Poa* which have not yet been analyzed molecularly. It also contributes to assessing the phylogenetic position of further equivocally circumscribed allies of Poinae, such as Dactylidinae, Cynosurinae, or Sesleriinae.

P1220. Molecular phylogeny of the Poaceae tribe Aveneae

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Phylogeny of the tribe Aveneae (Poaceae subfamily Pooideae) was examined using sequence data from the chloroplast gene *matK*. A broad sampling from the Aveneae was used, particularly taxa that were underrepresented in previous studies or proved difficult to place. The strict consensus tree shows that whereas current circumscription of subfamily Pooideae was confirmed, the delineation between Aveneae and Poeae was not. Some traditionally recognized subtribes of Aveneae or groups of genera commonly accepted as closely related were not resolved. Additionally, some large Aveneae genera appear as polyphyletic assemblages, e.g., *Helictotrichon* segregates into at least three different genera. The phylogenetic position of subtribe Duthieinae, usually considered to encompass the most "primitive" extant genera of Aveneae, will be discussed in relation to other Aveneae and to Stipeae.

P1221. Revision of the genus *Festuca* L. (Poaceae) in Italy

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The beginnings of modern systematic studies on the genus *Festuca* in Europe can be considered the *Monographia Festucarum Europaeorum* (Hackel 1882). For this reason an intensive study of the Hackel Herbarium in Wien (W) was started. In his systematic conception, the genus includes a modest number of species (28) articulated in a large number of infraspecific *taxa*. Following this work, nearly one hundred years later, the total number of species, reported by Markgraf-Dannenberg (1980, in *Flora Europea*) had increased from the 28 reported by Hackel, to 170, but this increase was not only due to the recognition of new entities, but simply to the raising to the rank of species of many infraspecific *taxa*. In Italy, unlike France or Spain, during the last century, systematic investigations on this genus are few. Since 1996, our research team has carried out taxonomic investigations on the genus *Festuca* in Italy, resulting in significant taxonomic rearrangements within critical groups and raising the number of *taxa* from 68 (reported in *Flora d'Italia*, Pignatti 1982) to roughly 100 entities.

P1222. Some results of taxonomy analysis of genus *Festuca* L. in flora of the Ukraine

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In the course of 1995 on 2004 analysis of systematic of genus *Festuca* in flora of the Western Ukraine (Polesye, Podolia, Carpathian mountains) has been undertaken. By the example of 130 populations of 15 species the anatomy-morphological differentiation narrowleaved festuque was investigated. About 5000 anatomic sections of leaf blades have been accomplished as well as measured 20 morphological parameters from which half is anatomic. Researches display that the existing biodiversity is far beyond traditional frameworks of species. However, no reason to describe new "fine" taxon because of continual nature of variability. The mass material has allowed to reveal geographical and ecological specialization of species. At the same time the wide polymorphism in the majority of species does not allow to mark out races precisely.

We share the point of view that in structure *F. valesiaca* agg and very heterogeneous *F. rubra* agg should be interpreted as subspecies. It is planned to expand region of investigations with application of new methods of researches. For today the specialized collection from almost 250 populations *Festuca* is created.

P1223. The origins and systematics of arctic polyploid alkali grass (*Puccinellia*; *Pooideae*; *Poaceae*) based on ploidy level and ITS sequences

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Alkali grass (*Puccinellia*) is a common pioneering grass in coastal and alkaline areas of arctic and temperate regions. To test whether polyploid origins underlie traditional problems of species delimitation in the genus, we studied three putative arctic polyploid species comparing them to the five Nearctic diploid species. We measured ploidy level by chromosome counts and flow cytometry and analyzed the internal transcribed spacer (ITS) region by sequencing and analysis of restriction sites diagnostic for the diploid species. Our results suggest that *P. bruggemannii* may be an auto-tetraploid of the diploid *P. vahliana*, *P. angustata* an allo-hexaploid of the diploid *P. arctica* and an as yet unidentified parent, and *P. andersonii* an allo-octoploid with at least two of the same parents as *P. angustata*. Although evidence suggests that the latter two species have the same set of parents, multivariate analyses of data from field specimens and a common garden experiment suggest they are morphologically distinct. Yet, occasional octoploids have the morphology of *P. angustata* in the field, but change to the morphology of *P. andersonii* in the common garden.

P1224. Systematics of *Dissanthelium* Trin. (Poaceae: Pooideae)

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Dissanthelium, with about 20 uncertainly delimited species, is characterized by two florets per spikelet and glumes that generally exceed the florets. The genus is distributed at high altitudes in the central Andes of South America with exception of two species in North America. The position of *Dissanthelium* within subfamily Pooideae is unclear. Clayton & Renvoize (1986) placed it in tribe Aveneae along with other long-glumed taxa, whereas all other authors have placed it in Poeae. Using DNA sequences from the nuclear ribosomal ITS and chloroplast *trnT-L-F* and *rpoB-trnC^{GCA}* regions, we are investigating the phylogeny of *Dissanthelium* and its position in Pooideae. Results show that the genus is related to members of Poeae, specifically *Poa*, and that its long glumes represent a homoplastic character. In addition, in each phylogeny *Dissanthelium* is not monophyletic and its species are nested within *Poa*. However, most of the species form a clade, and the remainder are related to Andean gynomonocious species of *Poa*. In addition to molecular data, we are gathering morphological (including micromorphology of the leaf and lemma), anatomical, and cytological data.

P1225. Molecular phylogenetic study of *Colpodium versicolor* and some other Aveneae and Poeae species.

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There are only few species of plants which have a remarkable low number of chromosomes, such as $2n=4$. They are *Haploappus gracilis* (Asteraceae; Astereae) and two species of grasses, *Zingieria biebersteiniana* (Poaceae; Aveneae) and *Colpodium versicolor* (Poaceae; Poeae). To study the origin of unusual two-chromosomes genome of *Colpodium* and *Zingieria* we have sequenced the internal transcribed spacers ITS1 and ITS2 as well as nuclear 5.8S rRNA genes of *Colpodium versicolor* and some other species of Aveneae and Poeae. The comparative analysis of ITS1+ITS2 sequences showed low genetic distance (p-distance) between *Colpodium versicolor* and *Zingieria biebersteiniana* and revealed strong support for the monophyly of these grasses genera. These results suggest that the reduction of chromosome number in the process of chromosome translocations took place in the chromosome complement of a common ancestor of *Colpodium* and *Zingieria*. This work was supported by the grant RFBR 03-04-49477.

P1226. Phylogeny of tribe Aveneae Dumort. (Pooideae, Poaceae) inferred from nuclear and chloroplast sequence analysisA. Quintanar¹, S. Castroviejo¹, P. Catalán²;¹Real Jardín Botánico, Madrid, Spain, ²Universidad de Zaragoza, Zaragoza, Spain.

Classical circumscription of tribe Aveneae has been evaluated through phylogenetic analysis of nuclear (ITS) and chloroplast (*trnT*) data across 145 representatives of avenoid genera and of the main lineages of its sister tribe Poeae. Parsimony and Bayesian inferences concur in depicting a congruent topology for the 'Aveneae core group' that shows the sister relationships of Agrostidinae to the Koeleriinae/Aveninae clade, each group characterized by contrasting morphological features. The Aveneae clade is also formed by the close lineages Anthoxanthinae, Brizinae, and Phalaridinae. Several genera traditionally included within Aveneae are resolved either in an intermediate placement between the Aveneae and Poeae (Poinae) clades (*Avenula*, *Deschampsia*, *Airinae* and *Holcus*) or embedded within the Poinae clade (*Alopecurus*, *Antinoria*, *Beckmannia*, *Cinna*, *Dielsiochloa*, *Milium*, *Phleum*, *Ventenata* and others). Traditional diagnostic traits seem to be inadequate to classify 'natural groups' within the Aveneae-Poeae complex. Considering potential reticulation events, morpho-anatomical and molecular characters should be reevaluated.

P1227. The importance of leaf anatomical data in the characterization of *Anthoxanthum aristatum* Boiss. and *A. ovatum* Lag. (Poaceae) in sympatric populations

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The genus *Anthoxanthum* L. includes several annual *taxa* that grow in dry and open habitats of S Europe and N Africa. Two of these species, *A. aristatum* and *A. ovatum*, can be found in sympatry in the Doñana National Park (SW Iberian Peninsula). Moreover, infraspecific *taxa* have been described in this area whose characterization remains controversial.

In this study we analyse the usefulness of anatomical data to differentiate these *taxa* when growing in mixed populations. Also, we try to evaluate the influence of environment on the anatomical traits, comparing these results with those obtained in allopatric populations. To attain these aims, 70 specimens from 6 populations were sampled and classified into taxonomical units. Subsequently, 22 anatomical leaf traits were measured. The similarity among samples was determined using the Phi coefficient and different multivariate analysis were applied to establish the relationships among samples (PCO and UPGMA clustering methods). To assess the influence of the environment on these traits CCA analysis was performed. Finally, the accuracy of some infraspecific *taxa* defined for these species has been analysed.

P1228. Problems of species of genus *Festuca* from ser. *Festuca* (*F. ovina* agg.) in Central Europe

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The treatment of selected problems in the *Festuca ovina* - group in Central Europe is given. This group includes four species - *F. eggleri* Tracey, *F. supina* Schur, *F. filiformis* Pourret and *F. ovina* L., the latter being probably divided into two subspecies: the diploid subsp. *ovina* and the tetraploid subsp. *guestfalica*. Some morphological, anatomical and cytological characters were collected and analysed. A comparison of some morphological and anatomical characters among these species is given. Two ploidy levels, 2n=14+0-5B corresponding to *F. ovina* subsp. *ovina* and 2n=28 corresponding to *F. ovina* subsp. *guestfalica* and *F. cf. duernsteinensis* were confirmed in *F. ovina*. The diploid chromosome number was found in *F. filiformis* Pourret and the tetraploid chromosome number was found in *F. supina* Schur. Two ploidy levels, diploid 2n=14 and tetraploid 2n=28 were recorded in *F. eggleri* Tracey.

P1229. Phylogenetic studies and the radiation of the New and Old World festucoids (Loliinae, Pooideae, Poaceae)L. A. Inda¹, J. G. Segarra-Moragues¹, P. M. Peterson², J. Müller³, P. Catalán¹;¹University of Zaragoza, Huesca, Spain, ²Smithsonian Institution, Washington, DC, United States, ³University of Jena, Jena, Germany.

We conducted an independent and combined phylogenetic analysis of ITS and *trnT* sequences on 168 representatives of New and Old World festucoids (Loliinae) to characterize biogeographical patterns. Nuclear and plastid derived trees using either parsimony or Bayesian algorithms support the major lineages and depict a monophyletic Loliinae. Ancient broad-leaved groups and the more recently evolved fine-leaved (FEVRE) clade have diploid species aligned basally. Diploid lineages distributed in the Mediterranean and Macaronesian regions likely gave rise to polyploid radiations in the Eurasian holartic region, Central and South America, and New Zealand. Within the FEVRE group, New Zealand taxa fall within the *Festuca* sect. *Aulaxyper* s.l. clade that incorporates the Macaronesian taxa and an American subclade. *Hellerochloa* and *Dielsiochloa* are included in the American subclade. *Vulpia* is polyphyletic since Euromediterranean and American species are derived from within different clades. Across the broad-leaved festucoid lineages American and Eurasian taxa are united in several subgeneric clades whereas the New Zealand taxa form a separate clade.

P1230. Systematic of Poaceae: an indication of gene duplication of GBSSI

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Monophyly of Poaceae is well supported in most studies, with strong support for three early-diverging lineages, and the PACCAD clade, but only moderate support for a clade including rices, true bamboos, and bluegrasses. Most subfamilies are well supported primarily based on chloroplast data; however, relationships among subfamilies are largely still unresolved, especially within the PACCAD clade. Of the nuclear markers used to date (PHYB, GBSSI, ITS), PHYB is the only one with good resolution. Previous GBSSI analyses generated an odd topology, which was interpreted as the result of poor sampling. In this preliminary analysis increased taxon sampling for almost all subfamilies recovered again an odd topology in which several subfamilies within the PACCAD clade appear polyphyletic. Several sequences and the polyphyletic nature of some subfamilies within the clade provide a hint of a possible duplication event of the GBSSI gene that happened perhaps near the base of the PACCAD clade. Increased clone sampling and southern hybridization analyses are in progress to provide additional data about this possible gene duplication event.

P1231. Partial incongruence between nuclear ribosomal ITS and chloroplast *trnT-trnF* phylogenies of the bluegrass genus *Poa* and allied genera: new insights into reticulate evolution in PoaceaeL. J. Gillespie¹, A. Archambault¹, R. J. Soreng²;¹Canadian Museum of Nature, Ottawa, ON, Canada, ²Smithsonian Institution, Washington, DC, United States.

Poa, known for taxonomic difficulty, polyploidy and apomixis, is widely presumed to have evolved reticulately. Comparison of phylogenetic analyses of nuclear and cpDNA sequences illuminated both ancient and recent reticulation events, while deep structure in the genus is largely stable. *Poa* is monophyletic if subg. *Andinae* is excluded and several small Poinae genera are included. Five major *trnT* clades diverge in the order: 1) subg. *Arctopoa* and sect. *Sylvestres* (ArcSyl); 2) sects. *Bolbophorum*, *Alpinae*, *Ochlopoa*, *Parodiochloa* (BAPO); 3) sects. *Secundae*, *Pandemos*, *Oreinos*, *Stenopoa*, *Tichopoa*, *Abbreviatae* (SPOSTA); 4) sects. *Poa*, *Macropoa* (PoM); and 5) sects. *Homalopoa*, *Madropoa*, *Dioicopoa* and related sections (HAMBADD). Taxa with different positions in the ITS analysis include: *P.* subg. *Arctopoa* outside of *Poa* with other Poinae and Alopecurinae; *P. trivialis* (sect. *Pandemos*) in BAPO; and the polyploid *P. abbreviata* in HAMBADD while diploid species of sect. *Abbreviatae* remained in SPOSTA.

P1232. Molecular phylogeny and divergence times of the A-genome species in *Oryza*: implications for origin of the cultivated rice

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The A-genome group in *Oryza* consists of eight diploid species and is distributed worldwide. The evolutionary relationships among species in this group have long been controversy and not well studied on sequence level mainly because of the lack of sufficient

resolution for commonly used sequences. We sequenced introns of five nuclear genes located on different chromosomes for 37 accessions representing two cultivated and six wild species from the A-genome group. As expected, the intron sequences provide much higher (more than two times higher) informative characters than those of ITS. Phylogenetic analyses by Maximum parsimony and Bayesian inference indicated that all the species except for *O. rufipogon* and *O. nivara* formed a monophyletic group and the Australian endemic *O. meridionalis* was the earliest divergent lineage. Two subspecies of *O. sativa* (ssp. *indica* and ssp. *japonica*) formed two separate monophyletic groups, suggestive of their polyphyletic origin. Based on molecular clock approach, we estimated that the divergence of the A-genome group occurred ~ 2.0 MYA while the two subspecies (*indica* and *japonica*) separated ~ 0.4 MYA.

P1233. Triba Stipeae in Siberia.

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Triba Stipae is represented in Siberia by 3 genus; *Achnatherum*, *Stipa* and *Ptylagrostis*, the most numerous and complicated to be *Stipa* (21 species), while *Ptylagrostis* (4) and *Achnatherum* (3). The species of *Stipa* are distributed in 5 sections, the largest to be *Stipa* (8 species), *Leiostipa* (6), *Barbatae* and *Smirnovia* contain in 3, and *Pseudoptilagrostis* 1 species. The study has shown, that most of *Stipa* species are quite variable. The study of variability of Siberian species showed that the greatest biodiversity is founded within the South Siberian mountains. The study of its distribution has shown, that it is limited in Eurasian continent. Most of species are of Euroasian distribution (57.14%), Asian group is less (38.10%). *Stipa barchanica* is endemic of S Siberia. Most of sections are represented mainly by Eurasian species, *Leiostipa* comprises Eurasian and Asian species in equal parts, *Smirnovia* and *Pseudoptilagrostis* contain only the Asian ones. 2 Siberian species of *Achnatherum* belong to Eurasian group, and 1 is Asian. All species of *Ptylagrostis* are Asian, 1 to be S Siberian endemic. Such a distribution is caused by the peculiarity of Siberian steppes forming.

P1234. Morphometry of the *Festuca valesiaca* agg. in Istria with the emphasis on *F. illyrica* MARKGR.-DANNENB. delimitation

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Possibility of recognition of the species *F. illyrica* MARKGR.-DANNENB. in the flora of Istria was studied. Precise analysis of species descriptions and the determination key, as well as field work have showed that there are no clear borders between this species and other members of *F. valesiaca* agg. In the analysis 316 individuals were taken into account and 20 characters were investigated. The data were analysed using multivariate and univariate statistical methods. It was possible to recognise only two groups corresponding with the species *F. rupicola* HEUFF. and *F. valesiaca* SCHLEICH. ex GAUDIN. Due to overlappings in the diagnostic characters with *F. rupicola*, the species *F. illyrica* could not be recognised as independent entity. The recognition between the species *F. rupicola* and *F. valesiaca* is possible only based on quantitative characters. The most discriminative character in delimitation of *F. rupicola* and *F. valesiaca* was the length of stommata.

P1235. Representatives of the genus *Alopecurus* L. (*Poaceae*) in Armenia

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The genus *Alopecurus* L. in Armenia is represented by 9 species from 4 sections. Sect. *Alopecurus*: *A. arundinaceus* Poir., *A. armenus* (K. Koch) Grossh.; Sect. *Colobachne* (Beauv.) Griseb.: *A. textilis* Boiss subsp. *textilis*, *A. textilis* subsp. *tiflisiensis* (Westb.) Tzvel., *A. brevifolius* Grossh., *A. tuscheticus* Trautv., *A. aucheri* Boiss., *A. laguroides* Bal. (= *A. dasyanthus* Trautv.); Sect. *Alopecurium* Dumort.: *A. aequalis* Sobol.; Sect. *Pseudopharis* Tzvel.: *A. myosuroides* Huds. According to the obtained data the feature of palea presence of *A. tuscheticus* (Aragats and Gegham floristic regions of Armenia) is very unstable in all Caucasian area (often palea is short, transparent or absent). *A. brevifolius* (Gegham, Darelegis, and Zangezour floristic regions) is very close

to *A. textilis*, differing by habitus, lower part morphology, lemma shape. An additional diagnostic feature (lower cauline leaves congestion) is revealed for *A. arundinaceus* and *A. armenus*.

P1236. Comparative morphological, histological and molecular studies of Hungarian wheatgrass (*Elymus* and *Agropyron*) taxa

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Comparative morphological, histological and molecular studies were carried out on four Hungarian wheatgrass species (*Elymus elongatus*, *E. repens*, *E. hispidus*, *Agropyron pectiniforme*) and the energy grass 'Szarvasi-1'. The cultivar, developed in Hungary, has gained more importance recently as a renewing source of energy. The morphological features of the roots, the culms, the leaves and the spikes were described in detail. The histology of the culm and the leaf was also studied, describing the relative position of the vascular bundles and the sclerenchyma in the internodes and nodes, as well as the leaves. The proportion of different tissues was determined in each studied organ, in relation to the dry matter content.

Taking into consideration the standpoints of land utilization, economicalness and environment protection, it is important to clarify the systematic position and relationships of the taxa. DNA level studies (rpoA gene of plastid genome) made it possible to recognize not only the cultivar but also the *Agropyron* genus with debatable taxonomic situation. Phylogenetic analyses and bootstrapping were performed using PAUP (ver. 4.0; Swofford, 2002).

P1237. Biosystematic studies of the *Elymus repens* (L.) Gould (*Poaceae*, *Triticeae*): evidence from morphology, anatomy and AFLPs.

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Combined morphological, anatomical and molecular approaches were used to unravel variation within *Elymus repens*. Forty four populations were analyzed for 49 morphological and 26 anatomical characters. Multivariate morphometric analyses (PCA, ANOVA and CDA) provided significant support for recognition of three varieties of *E. repens*: var. *repens*, var. *aristatus* and var. *subulatus*. Genetic infraspecific diversity was assessed by Amplified Fragment Length Polymorphisms (AFLPs). Twenty AFLP multilocus phenotypes were observed in the 70 specimens analyzed, based on 104 (37.28%) polymorphic AFLP markers. The analyses of molecular variance (AMOVA) showed significant genetic differentiation among populations, but not significant among varieties. The low levels of AFLP variation indicated that conspicuous morphological variation of *E. repens* is caused by plasticity, somatic mutations and habitats changes. *E. repens* is an evolutionarily young species, of hybrid origin, in which microevolutionary processes continue. New combination was made: *Elymus repens* (L.) Gould var. *subulatus* (Roem. & Schult.) Szczepaniak, comb. nova.

P1238. Phylogenetic analysis of the "Briza Complex" (*Poaceae* - *Pooideae*)

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The genus *Briza* L. comprises 4 to 23 species, depending on the criteria adopted by the different authors. *Briza stricto sensu* includes only euroasiatic species, but considered in a broad sense it includes also the South American species, alternatively placed in genera like *Calotheca* Desv., *Chascolytrum* Desv., *Microbriza* Parodi ex Nicora & Rúg., *Lombardochloa* Roseng. & Arr., *Poidium* Nees and *Rhombolytrum* Link. The circumscription of the so called "Briza Complex" remains controversial, although some analyses based on morphological data were already published, without solving the delimitation problems. The present work includes a phylogenetic analysis of this complex, based on both morphological and DNA data. Twenty seven morphological characters and three totally or partially sequenced DNA fragments were included: nuclear ITS, chloroplast *trnL* intron and *trnL-trnF* spacer. Parsimony and Maximum Likelihood analyses were performed with PAUP *, using distinct or combined matrices. The preliminar analyses confirm *Briza stricto sensu*, with strong

bootstrap support, and an American clade, including all other genera.

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P1239. Revision of the genus *Festuca* L. in Southern Transcaucasia

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The genus *Festuca* L. is represented in Southern Transcaucasia (Armenia and Nakhichevan) by 16 species belonging to 4 following subgenera. Subgen. *Drymanthele* V.I.Krecz. & Bobrov: *F. drymeja* Mert. & W.D.J.Koch, *F. altissima* All. Subgen. *Leucopoa* (Griseb.) Tzvelev: *F. sclerophylla* Boiss. ex Bisch. Subgen. *Schedonorus* (Beauv.) Peterm.: *F. gigantea* (L.) Vill., *F. pratensis* Huds., *F. arundinacea* Schreb. Subgen. *Festuca*: *F. woronowii* Hack. (with 2 subspecies: *woronowii* and *caucasica* (St.-Yves) E.B.Alexeev), *F. skvortsovii* E.B.Alexeev, *F. chalcophaea* V.I.Krecz. & Bobrov, *F. ruprechtii* (Boiss.) V.I.Krecz. & Bobrov, and 6 species belonging to *F. aggr. valesiaca* Schleich. ex Gaudin: *F. valesiaca* Schleich. ex Gaudin, *F. brunnescens* (Tzvelev) Galushko, *F. oreophila* Markgr.-Dann., *F. vagrariviva* E.B.Alexeev, *F. saxatilis* Schur, *F. karsiana* E.B.Alexeev (reported for the first time from S.Transcaucasia (N.Armenia)). The presence of *F. rubra* L. in the region isn't confirmed.

P1240. Evaluation of genetic diversity in some bread wheat cultivars using SSR marker

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In the present study microsatellite markers were used for germplasm analysis and estimation of the genetic relationship and diversity between 21 genotype of persian breed wheat. 37 microsatellite markers, developed from *Triticum aestivum* sequence were selected for the analysis. The number of alleles per microsatellite marker varied from 2 to 10 (average 5.37) and a total of 199 alleles were detected. Gel scoring was with present(1) and absent(0) band. That was studying genetic diversity(DI) and polymorphism information content(PIC) and with average was 0.68 and 0.63 respectively. Genetic similarity was calculated with Nei and Lee and Jaccard and Simple Matching methods with average 0.201 and 0.325 and 0.725 respectively. The highest levels of genetic similarity for genotypes were found in Ghods and Alnad genotype in the tree methods and lowest was in Bolani and Niknejhad with Nei and Lee and Jaccard methods but in Simple matching methods were in the Shain and Tajan cultivars. The result showed that all of the genotype could be distinguished and clustered into 4 groups with nei and lee similarity method and UPGMA clustering method with programme NTSYS.

P1241. Cyto-Morphological study of some *Stipa* (Poaceae) species and populations in Iran

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Morphometric and cytological studies were performed on several populations of Iranian *Stipa* species concerning polyploidy level, chiasma frequency and distribution, chromosomes association and segregation. The species and populations studied possessed 2n = 24, 36 and 44 chromosome number. The chromosome numbers of four species are reported for the first time. The populations and species studied differed significantly in their meiotic characteristics. Meiotic abnormalities observed included laggard chromosome formation, stickiness and cytomixis. Cytomixis led to the formation of aneuploid meiocytes. Unreduced pollen grains were observed in some of the species, which differed significantly in their size compared to the normal (reduced) pollen grains. Cluster analysis and ordination plot of the species studied based on morphometric and cytological characters supported Freitag taxonomic treatment of the group. Detailed description of the species is provided using statistical analysis.

P1242. Contrasting morphology and molecules in *Bouteloua media*, a polyphyletic species in the *B. curtipendula* clade (Gramineae, Chloridoideae).

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Bouteloua media is the tallest, most robust species in the *B. curtipendula* complex, a taxonomically difficult, mostly North American clade. Although 11 species are generally recognized in the complex, species circumscription is not clear cut. *Bouteloua media* is distributed from tropical Mexico to South America. In addition to size, the species is distinguished by a firm, knotty base, frequently pubescent leaves, lateral (axillary) inflorescences, many branches per inflorescence, and many spikelets per branch. Molecular phylogenies (ITS and *trnT-L-F*) of the complex show that representatives of *B. media* from southern Mexico, Central America, and South America are nested in the earliest-diverging clade, whereas plants from central and southern Mexico are positioned in the most derived clade. Sympatry of plants representing the northern and southern lineages occurs in Oaxaca, Mexico. The congruent phylogenies suggest that the morphological characters used to circumscribe this polyphyletic species are homoplasious, and that non-molecular, phylogenetically congruent characters should be sought out for taxonomic revision.

P1243. A genealogical approach reveals the impossibility of correct phylogenetic inference from chloroplast data in *Hordeum* (Poaceae)

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To analyze reasons for inconclusive results of earlier chloroplast phylogenies in the grass genus *Hordeum* we established a genealogy of chloroplast haplotypes by sequencing the *trnL-F* region in 800 individuals, covering all 31 species of the genus. While the outcome of phenetic and parsimony analyses of 88 haplotypes were ambiguous, the network approach showed that in *Hordeum* ancient chloroplast types co-occur with their descendants. Moreover, we found up to 17 chloroplast haplotypes within single species and up to 7 species sharing single haplotypes. Ancient polymorphisms together with incomplete lineage sorting occurred preferentially in the rapidly speciating New World taxa of the genus, where shared chloroplast types have persisted for at least four million years. Far reaching extinction in Europe and the survival of chloroplast lineages in Asia and North America resulted in a phylogenetic tree that pronouncedly differs from nuclear estimations of species relationships. Thus, even for the deepest splits in the genus, reaching back more than nine million years, no safe phylogenetic inference from chloroplast data is possible in *Hordeum*.

P1244. Phylogeny of Chloridoideae (Poaceae) based on *ndhF* sequences (cpDNA)

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Chloridoideae (Poaceae) are a well-supported clade comprising ca. 140 genera and 1500 species distributed in the tropics and subtropics. The subfamily contains a few large and many small and monotypic genera. Analysis of *ndhF* sequences from 58 genera and 73 species, representing all tribes (*sensu* Clayton and Renvoize 1986), places *Triraphis mollis* as sister to three major clades. The next diverging lineage includes the large genus *Eragrostis*, Unioliinae, and Pappophoreae (excluding *Pappophorum*). The third diverging clade includes *Spartina*, the large genus *Sporobolus*, and *Zoysia*. The remaining clade includes most of the genera within the subfamily, including *Chloris* and relatives, *Bouteloua*, *Muhlenbergia* and *Pappophorum*. All tribes excluding Orcuttieae (three genera) and Leptureae (one genus) are not monophyletic. However, morphological synapomorphies have been identified for most of the major clades. The *ndhF* phylogeny suggests considerable homoplasy in inflorescence form, a character that has been widely employed in grasses for tribal circumscription.

P1245. Historical retrospective, systematic and biogeography of *Leandra Raddi* (Melastomataceae) from Brazil.

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Leandra, with ca. 250 spp., is distributed from southern Mexico and Antilles to the northern Argentina. The last revisions of this

group dates of the 19th century and it commonly presents taxonomical complex and several herbaria specimens are identified as species of another genus. It has been recognized as monophyletic group and closely related to *Clidemia*, *Miconia* and *Ossaea*. This project presents a retrospective of *Leandra* history, standing out the classifications and the fragility of the sections circumscriptions and propositions of new synonyms and species. *Leandra* is characterized mainly by the terminal and pseudo-axillary inflorescences, the floral buds with acute to acuminate apex, the petals with attenuate-acuminate to -acute apex and fleshy fruits of the bacidium type, polyspermic. In Brazil, it is represented by ca. 200 spp. that occur in different ecosystems, from sea level to high and cloud forests, in tropical rain forests. The largest concentrations of species are in the Southeast (73%) and South (34%) regions, and the smallest is in the Central region (4%). There are 166 spp. restricted to Brazil and from these 83 represent punctual endemism.

P1246. Structure of the puzzling female inflorescence of *Amphipterygium* and comparison of the flower structure with *Pistacia* (Anacardiaceae)

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Amphipterygium and relatives were originally placed in their own family Julianiaceae mainly because of their unique female inflorescences. Later, overall structure, wood anatomy and flavonoid chemistry prompted their inclusion in Anacardiaceae, and this was supported by molecular phylogenetic studies. Our aims were (1) to reveal the enigmatic structure of the female inflorescence and (2) to compare the female flower and its bizarre ovule with similar ones in other Anacardiaceae. The female inflorescence is a few-flowered thyse comprising regularly one or two cymose units, each of which develops into a samaroid structure. The flattened part is the peduncle and the globose part is an involucre. The involucre is almost closed and contains three to five collateral flowers but only one will produce a seed. During development, the flower bases are deformed to a saddle-like shape by the differential growth of the basal inner part of the involucre. The unitegmic ovule with two lateral and one large basal appendage is highly similar to that in *Pistacia*. The placement of *Amphipterygium* in a clade with *Pistacia* is also highly supported by other reproductive features.

P1247. Taxonomic revision of *Galipea* Aubl. (Rutaceae, Galipeae)

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The neotropical genus *Galipea* Aubl. is marked by its carpels connate and fruit syncarpous before dehiscence, by a long and thin corolla tube, and by its androecium with only two fertile stamens, bearing anthers that are sterile at base above point of attachment to filament, appendaged at base, connate by their sterile bases and appendages. A morphological and taxonomic revision of the group lead to the recognition of 16 species, from which 7 are new, with *G. carinata*, *G. congestiflora*, and *G. revoluta*, all from Brazil, already published in 2004. The species can be separated by vegetative and reproductive characters, expressed in an identification key. Morphological descriptions, and comments on habitats, geographic distribution, taxonomic relationships and variability are presented. Although the distribution of the genus extends from Costa Rica in Central America to Brazil and Bolivia, all species show restrict distribution areas, some of them being narrow endemics.

P1248. Phylogeny of *Eucalyptus* subgenus *Eudesmia* based on molecular and morphological evidence

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Eucalyptus (family Myrtaceae) is a ubiquitous genus of the Australian landscape. *Eucalyptus* subgenus *Eudesmia* contains 26 taxa, in tropical and arid regions of Australia. Stellate hairs, pith glands, stamens usually in four bundles, and free sepals on the buds and fruit of most taxa traditionally define the subgenus. A molecular phylogenetic parsimony analysis is presented based on the *psbA-trnH* region of the chloroplast genome, and the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA. Morphological characters of trichomes, seeds, fruit, flowers, seedlings and fruit wall anatomy were also coded and analysed.

Three main clades with strong bootstrap support were resolved: 1 *E. baileyana* - *E. tetradonta* clade (4 taxa), 2 *E. miniata* clade (5 taxa), and 3 *E. roycei* - *E. erythrocorys* clade (17 taxa). Clades 1 and 2 are distributed in northern Australia from the Kimberley to eastern Queensland, and clade 3 is distributed in southwest Western Australia and deserts.

P1249. Phylogeny of *Pilocarpus* (Rutaceae) and stochastic mapping of characters

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Pilocarpus is a neotropical genus of 17 species distributed from Mexico to Brazil and Argentina. Differently from its allied genera, *Pilocarpus* is marked by its racemes and anthers bearing a dorsal gland. We investigate the phylogeny of *Pilocarpus* using a set of 104 morphological characters, partitioned into four subsets (leaf, flower, fruit, and others) to allow across-partition rate independence. Symmetric Markov models were assumed as priors for all characters and computer simulations were performed to address both mapping and phylogenetic uncertainty. We found that: (1) *Pilocarpus* is highly supported as monophyletic, with *P. microphyllus* being sister to all other species; (2) The simple-leaved species plus *P. pauciflorus* (a species with unifoliate compound leaves) form a clade; (3) The species with pinnately compound leaves are not monophyletic; (4) The species with versatile anthers and superposed ovules form a moderately supported clade. Computer simulations showed different degrees of asymmetry among characters (specially within the leaf and flower partitions), and suggest a potentially directional evolution for some of them. FAPESP

P1250. Phylogenetic relationships of *Begonia* (Begoniaceae) with emphasis on the Chinese species: evidence from ITS

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Begonia is one of the most diversified genera in angiosperm, with ca. 1400 species in 65 sections in Africa, Asia, and America. About 150 species in 9 sections occur in China. Phylogenetic analysis of 156 ITS sequences representing 148 taxa from 36 sections (70 taxa in 7 sections from China) from all continents was conducted to elucidate the phylogenetic relationships within the genus. The resulting trees show that the African taxa form a series of basal branches and the Asian species are divided into several unequal clades successively diverged after the African taxa. Species from America and S. Africa form a clade sister to a large, poorly resolved Asian clade containing most species from sections *Platycentrum*, *Diploclinium*, *Reichenheimia*, *Sphenanthera*, and *Leprosae*. The Chinese species do not group according to their sections. None of the more well-sampled sections in China is suggested to be monophyletic. Possible rapid diversification in mainland Asian *Begonia* is inferred. The results suggest an African origin of *Begonia* and an Asian origin of the American-S. African clade. Reevaluation of infrageneric classification is desirable.

P1251. Morphological phylogeny of *Cucumis-Cucumella* (Cucurbitaceae)

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A phylogenetic hypothesis was developed for 44 *Cucumis-Cucumella* species using 122 morphological characters. *Oreosyce africana* and *Mukia maderaspatana* served as outgroups. Qualitative and quantitative characters yielded 104 trees with 967 steps, consistency index (CI)=0.3681, and homoplasy index (HI)=0.6319, and qualitative characters alone yielded 8 trees with 805 steps, CI=0.3801, and HI=0.6199. *Cucumis* was polyphyletic, and *Cucumella* paraphyletic, sensu Farris. *Cucumella* species were transferred to *Cucumis*, making it a monophyletic group. When the *Cucumis-Cucumella* species were reanalyzed with the known hybrid *Cucumis* × *hytivus*, it had little effect on the phylogenetic hypothesis, and its hybridity and parentage could not be deduced from the results. Aculei on the fruit, used as a defining character in all previous subgeneric classifications, are very labile, and have been lost eight times during species evolution. It is a

very useful character for species identification, but should not be used in the infrageneric classification.

P1252. Comparative carpology and systematic of the family *Malvaceae*

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The family *Malvaceae* is characterized by considerable variety of fruit types. There are typical polyfollicles with elongated receptacle, capsules, and disintegrated fruits, which split into monospermous mericarps. The anatomical structure of pericarp sufficiently differs in various fruit types in representatives of the family. Pericarp structure of different types of disintegrated fruits sufficiently differs and it is impossible to treat them as schizocarpic fruits. The ultrasculpture of fruit surface is also considerably varies. Basing on the analysis of received carpological data we have to conclude that *Malvaceae* should not be divided into tribes, as it is accepted in most systems. Our materials imply the necessity of subdivision the family into seven separate and well-defined by complex of carpological characters subfamilies. These families are *Malopoideae*, *Gossypioideae*, *Hibiscoideae*, *Malvoideae*, *Philippodendroideae*, *Sidoideae*, and *Urenoideae*.

P1253. A new classification for Onagraceae

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Onagraceae comprise a moderate sized but well-known plant family that is well-delimited within the order Myrtales, closely related to Lythraceae. Recent analyses of molecular sequence data support many aspects of the classification of Onagraceae derived from earlier morphological analyses, but suggest several changes, especially in the large tribe Onagreae. In particular, the genus *Camissonia* is not monophyletic, and is best treated as five generic lineages (*Camissonia*, *Chylismia*, *Chylismiella*, *Eulobus*, and *Taraxia*), each defined with one or more morphological synapomorphies. On the other hand, *Oenothera* is strongly supported as a monophyletic lineage, but only with the addition of several traditionally segregated genera. Thus, *Calylophus*, *Gaura*, and *Stenosiphon* all now fall within the expanded *Oenothera*, re-defined morphologically by presence of an indusium on the style and a lobed or peltate stigma. Tribe Onagreae appears to have arisen from northern Mexico, proliferating into either western North America (*Xylonagra*, *Clarkia*, *Gayophytum*, the *Camissonia* complex, and parts of *Oenothera*) or central and eastern North America (most of *Oenothera*).

P1254. Relationships among major clades of the expanded Malvales: the contribution of *ndhF* data.

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Relationships among major lineages of the expanded Malvales are poorly understood. Two previously published *rbcl* analyses showed very different topologies, both weakly supported. In Fay et al. (1998), the bixaceous clade (*Bixa*, *Diegodendron* and *Cochlospermaceae*) was basal; *Thymelaeaceae* and *Sphaerosepalaceae* together were sister to the rest (core Malvales and a clade containing *Dipterocarpaceae* and other families, including *Neurada*). In Alverson et al. (1998), *Neurada* was the basal lineage; *Sphaerosepalaceae* were sister to the bixaceous clade and *Thymelaeaceae* to the dipterocarp clade (excluding *Neurada*). To clarify the relationships of *Sphaerosepalaceae*, cpDNA sequences from relevant lineages were generated and obtained from GenBank. Preliminary analyses of the 5' portion of *ndhF* suggested that *Sphaerosepalaceae* were sister to the core Malvales, contradicting both *rbcl* trees. As with *rbcl*, the broader topology varied depending upon choice of outgroups and key branches were weakly supported, reinforcing the theme that rapid radiation followed by extensive divergence has hampered elucidation of phylogenetic history in this group.

P1255. Is dioecy ancestral in Cucurbitaceae and monoecy derived?

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Based on 10 loci from the three plant genomes, we earlier reported that Cucurbitales consist of (Anisophylleaceae ((Begoniaceae, Datisceae, Tetramelaceae [BDT]), (Coriariaceae, Corynocarpaceae)), (Cucurbitaceae)). A possible position of Cucurbitaceae (represented by 11 genera from all major lineages) as sister to the BDT clade has 74% jackknife support. The placement of Cucurbitaceae, and whether Fagales or Rosales are sister to Cucurbitales, greatly influences the inferred evolution of monoecy and dioecy, the typical sexual systems in Cucurbitales. With Rosales sister and Cucurbitaceae sister to BDT, dioecy is inferred to be ancestral in Cucurbitaceae, which fits with basal clades in the family indeed being dioecious. With the mainly monoecious Fagales sister to Cucurbitales, the ancestral sexual system in Cucurbitaceae becomes ambiguous because Anisophylleaceae and Corynocarpaceae/Coriariaceae are monoecious or perfect-flowered. Sexual systems in cucurbits are exceptionally labile, however, and parsimony may underestimate switches even if the true phylogeny were known.

P1256. Phylogenetic study of the polyphyletic genus *Leandra* (Miconieae: Melastomataceae), based on DNA sequence data:

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The Melastomataceae is a tropical family including around 4000 species that are divided into 12 tribes. The tribe Miconieae is strictly neotropical, and has close to 2000 species in 30 genera. Previous phylogenetic analyses of the Miconieae have shown that many genera are derived from within a paraphyletic *Miconia*. However these studies have only included six species of *Leandra* a genus of 250 species characterized by acute petals and terminal inflorescences. In spite of this limited sampling, it has been shown that *Leandra* is most likely not monophyletic. In this study we present results of an analysis that includes data from two loci (one nuclear, one plastid), with and expanded sampling for this genus. Even though *Leandra* is clearly polyphyletic, and embedded in the phylogenetic structure of *Miconia*, some distinct and well supported clades do exist. Some of these clades correspond to sections or groups previously recognized. One such group is composed by species from section *Tschudya*, which is characterized by seeds with an appendage. The monotypic genus *Platycentrum* (which at times has been recognized as *Leandra*) is recovered within another clade of *Leandra*.

P1257. Rosaceous evolution in the Okanogan Highlands, Washington, USA and British Columbia, Canada

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Examinations of the evolution of major angiosperm families often rely on a fossil record consisting of a few members being represented in widely disparate locations. However, in the case of Rosaceae, a major episode of the initial diversification of the family is documented in the Eocene upland floras of the Okanogan Highlands. These lake deposits of northeastern Washington, USA, and British Columbia, Canada contain well preserved compression material of flowers, fruits and leaves representing over a dozen rosaceous taxa. Among those recognized, are both extant (*Prunus*, *Amalanchier*, *Spirea*) and extinct (*Stonebergia*) genera of all four of the traditionally recognized subfamilies. The diverse floras at Republic, Washington and One Mile Creek and related localities in British Columbia provide a snapshot of a group undergoing major radiation. The Okanogan Highlands was a key segment of a migrational route for multiple exchanges of North American and Asian taxa. The complexity seen in Eocene Rosaceae suggests that microevolutionary processes documented in present-day members of the family may have been pivotal to early evolution of Rosaceae.

P1258. The genus *Rubus* L. (Rosaceae Juss.) in the flora of West Ukraine

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The genus *Rubus* in the flora of the West Ukraine is represented by 31 species. First time for the flora of West Ukraine it was adduced 6 species: *R. divaricatus*, *R. opacus*, *R. praecox*, *R. orthostachys*, *R. gothicus*, *R. haesitans* and subspecies *R. nensensis* ssp *scissoides*. From the list of blackberries of the

regional flora have been excluded *R. chamaemorus*, *R. macrophyllus*, *R. radula*, *R. carpinifolius*, *R. pyramidalis*, *R. vestitus*, *R. koehleri*, *R. kuleszae*, *R. camtostachys*, *R. mollis*, *R. serpens*, because of absence of herbarium specimens, that prove their location in the West Ukraine; their previous inclusion in the list of species was connected probably with incorrect definition. The botanical-geographical and ecological-geographical analysis of the species of the *Rubus* in flora of West Ukraine are carried out. Analysis of the present distribution of blackberries in West Ukraine gets an evidence that great part of found species are blind to Karpathians and Rostotche, where one of the diversity centrum of the *Rubus* in Ukraine is situated.

P1259. Molecular phylogeny and biogeography of the genera *Mespilus* L. and *Crataegus* L. (Rosaceae)

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Crataegus is one of the largest genera within Rosaceae subfamily Maloideae. We have used two nuclear (*ITS*, *LEAFY* intron 2) and four chloroplast (*trnG/S*, *psbA/trnH*, *trnH/rpl2*, and *rpl20/rps12*) regions to infer phylogenies with which to test the existing sectional classification of the genus. Our sample consists of 133 accessions representing 66 species of *Crataegus* in 15 sections from both the Old and New World. In addition, 18 accessions representing the two species ascribed to the closely related genus *Mespilus*, together with species of *Amelanchier*, *Malus*, and *Aronia* as outgroups, are included. Besides clarifying relationships between *Mespilus* and *Crataegus*, our results indicate that some sections (e.g. *Brevispinae*) may not be monophyletic. Strong biogeographic patterns are observed among sections: (1) the western North American section *Douglasii* is sister to the East Asian *Sanguinea*; (2) section *Crataegus* of western Eurasia is closely related to the Asian *Hupehensis* and *Cuneatae*; (3) the co-occurrence of several sections in eastern North America such as *Cordatae*, *Microcarpa*, *Coccinea*, *Crus-galli* and *Aestivales* is not due to their monophyly.

P1260. Intraspecific variability of European *Rosa* (sect. *Cinnamomeae*) and delimitation of the species.

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In Russian literature *Rosa majalis* is usually split into 4 to 5 separate species basing on fruit shape, indumentum, and glandulousness of various parts of the plant. Recently some of the European botanists seem to be inclined to support such a point of view, too. The analyses of infra- and interpopulational variability of these characters revealed that all the morphs described as species can occur within a single population. Numerous intermediates between them do exist. Neither clear pattern in geographic distribution of these morphs, nor a significant correlation between variable characters can be observed. Similar combinatory patterns of variability exist in related species *R. acicularis* and *R. alpina*. Delimitation of these species is possible by characters other than fruit shape and pubescence, such as growth form, leaflet number, shoot's armature, pedicel length, etc. Two neglected taxa of the sect. *Cinnamomeae* however occur in European Russia, mostly in cultivation. One of them differs from *R. majalis* in armature and flower characters and is of still unknown origin. The other is probably the Linnean *R. pendulina*, an early cultivar of N American *R. blanda*.

P1261. Promiscuity in the Andes: using AFLP and morphological data to examine species relationships in *Polylepis* R. & P. (Rosaceae, Sanguisorbeae)

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Polylepis R.&P. is a genus of small to large trees distributed along the Andes from Venezuela to Argentina. It is especially notable for including representatives reaching the highest elevations among woody plants worldwide. In the present study, AFLP and morphological data were used to reconstruct the phylogeny of the genus. Results from the morphological analysis correlate well with earlier hypotheses about evolution in the genus, showing a progression from large trees with numerous, thin-textured leaflets, and many-flowered inflorescences to reduced growth size, few, thick-textured leaflets, and few flowers per inflorescence. While a

basic phylogenetic signal is also discernible in the AFLP analysis, relationships at the tips of the AFLP trees seem to be dictated mainly by geographic proximity rather than systematic affiliation. This structure is interpreted as indicative of frequent hybridization and introgression, raising the question how species with extensive geographical ranges maintain their apparent morphological cohesiveness.

P1262. Bracteoles in *Crataegus* Systematics

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The inflorescence bracteoles of *Crataegus* (Rosaceae) were investigated to determine their value in hawthorn systematics. Studies to date have revealed: (1) two significantly different bracteole types occur, a symmetric kind found in all species and an asymmetric kind found only in Asiatic species; (2) there is considerable variability in texture, size, width, venation, and glandularity within the symmetric kind; (3) preliminary analysis of the data indicates the potential for a valuable elucidation of the existing taxonomy; (4) studies on their function found evidence to suggest that bracteoles in *Crataegus* are the site of extrafloral nectaries that serve to decrease the number of floral visits made by ants. Now that different species of *Crataegus* have been found to possess characteristic bracteole forms, bracteoles can begin to be used routinely in taxonomic keys. It is expected to have morphometric analyses available at the congress.

P1263. Phylogenetic relationships in tribe Spiraeae (Rosaceae) inferred from nucleotide sequence data

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Tribe Spiraeae has generally been defined to include *Aruncus*, *Kelseya*, *Luetkea*, *Petrophyton*, *Sibiraea*, and *Spiraea*. Recent phylogenetic analyses of Rosaceae have supported the monophyly of a group including those genera (although *Sibiraea* has not yet been sampled) plus *Holodiscus*, formerly classified in Tribe Holodisceae because fruits in this genus are achenes rather than follicles. *Spiraea*, with 50-100 species distributed throughout the north temperate regions of the world, is by far the largest and most widespread genus in the group. The remaining genera have one to several species each and most of them are confined to North America. Phylogenetic analyses of nucleotide sequences from the nuclear (ITS) and chloroplast (*matK*, *trnL-F*) genomes support monophyly of a group including *Kelseya*, *Petrophyton*, and *Spiraea*, which is sister to a clade including the other genera. Within *Spiraea*, none of the three sections defined by Rehder based on inflorescence morphology is supported as monophyletic. Phylogenetic analyses suggest that there have been several biogeographic events involving vicariance or dispersal between the Old and New Worlds within this group.

P1264. Taxonomy of The Old World *Crataegus* on The Model of The Turkish *Crataegus*

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Turkey is an important diversity center for the genus *Crataegus*. Section *Crataegus* because it lies in an area between the three main phytogeographical regions, namely, the Euro-Siberian, Mediterranean and Irano-Turanian regions. In addition to this, the country is characterized by a wide range of topographical structures and of vegetation cover, and diverse climatic features.

This study includes all the Old World *Crataegus* taxa. Both taxonomical and nomenclatural studies on the section have been continuing.

The most important problem for the taxonomy of the *Crataegus* taxa is probably hybridization. This has been frequently observed during the field studies in Turkey and several intermediate specimens have been collected by the present author. However, some of the taxa which have been formerly proposed as hybrid origin do not seem to be so according to field observations on their biogeography, flowering trait and other features. This study is intended to discuss the latest situation of the taxonomy of the Old World *Crataegus* taxa, some of the special problems, and possible future works for a better taxonomy of the genus.

P1265. Molecular diversity and phylogenetic position of the genus *Sibiraea* within the family RosaceaeT. Grebenc¹, D. Ballian², G. Boži¹, H. Kraigher¹;¹Slovenian Forestry Institute, Ljubljana, Slovenia, ²University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

Croatian sybirea (*Sibiraea croatica* Deg.) is a rare and endemic species described from western Balkan region, representing a tertiary relict in regional flora. Only a few data is known about the biology and genetics of this IUCN protected species. We have studied Croatian and Altaic sybirea (*S. altaicensis* (Laxm.) C. K. Schneider) by comparing the genetic diversity and differentiation of two *S. croatica* populations from the Balkan region with two populations of *S. altaicensis* from regions of southern Russia and Siberia. Based on the molecular analyses of ITS regions in nrDNA we propose Croatian sybirea only as an ecotype of Altaic sybirea, yet retaining its high need for protection. The observed low genetic diversity is also supported with only a few morphological differences between taxons. The phylogenetic analysis of ITS regions in nrDNA positioned sybirea into the tribe Spireae, equally to its position previously determined by morphological characteristics.

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P1266. First phylogeny of *Sabicea* and its allied genera inferred from ITS dataS. A. Khan^{1,2}, S. G. Razafimandimbison^{3,4}, B. Bremer^{3,4}, U. Meve¹, S. Liede-Schumann¹;

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Sabicea is the type genus of the tribe Sabiceae presently placed in the subfamily Ixoroideae (Rubiaceae). This pantropical genus has its centre of diversity in African mainland with 82 species, followed by the Neotropics with 63 species, Madagascar with 6 species, and São Tomé and Príncipe with 3 species. *Sabicea* has been circumscribed by a usually scandent habit, axillary inflorescences, valvate corolla lobes, (2-)3-5 locular ovary with numerous ovules in each locule, and fleshy fruits. Recognition of *Sabicea* as a separate genus has never been challenged, but its monophyly has never been tested using molecular-based phylogenies. In this first phylogenetic study of *Sabicea* and its allies we perform parsimony analyses based on ITS data to test the monophyly of *Sabicea*. Our analyses show that the current delimitations of *Sabicea* and *Pseudosabicea* do not form monophyletic units. Neotropical *Sabicea* is resolved as monophyletic, whereas African *Sabicea* seem to be para- or polyphyletic.

P1267. Phylogeny of the "*Hedyotis* group" (Rubiaceae)

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Phylogenetic studies have shown the tribe Hedyotideae is paraphyletic. The tribe Spermaceae is nested within the Hedyotideae. Some authors have suggested an emended Spermaceae to include these two tribes and the Knoxieae, Manettieae, and Triainolepideae. Other authors have suggested a separate Knoxieae and Spermaceae (Spermaceae, Manettieae, and "*Hedyotis* group"). The *Hedyotis* group is a polyphyletic assemblage of 41 genera and over 800 species. Further complicating the circumscription of the group is that the two largest genera *Hedyotis* and *Oldenlandia* are polyphyletic. This study used *trnL*, *rps16*, and ITS data to examine the phylogeny of the group using a broader, more comprehensive sampling. Preliminary results indicate that lineage-sorting has a strong correlation to biogeographic distributions. Genera such as *Arcytophyllum* and *Houstonia* are forming monophyletic lineages; however, other new groups are being resolved that are not defined by clear morphological synapomorphies.

P1268. Phylogeny and classification of Ixoreae (Rubiaceae: Ixoroideae)A. Mouly¹, B. Bremer², J. Florence³, J. Jérémie¹;

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The tribe Ixoreae, of the subfamily Ixoroideae s.l. (Rubiaceae), as presently circumscribed (Andreasen & Bremer 2000) contains ca. 400 species and 6 genera (*Captaincookia*, *Doricera*, *Ixora*, *Myonima*, *Scyphiphora* and *Versteegia*) shrubs and trees. The pantropical genus *Ixora* is the largest genus with 300 to 400 species, some of which are commonly cultivated as ornamentals throughout the world. The monophyly of its current circumscription has never been tested before. The authors perform phylogenetic analyses using sequencing data from two chloroplast (*rps16* and *trnT-F*) and one nuclear (ETS) markers and morphological data to test the monophyly of *Ixora* and Ixoreae. The study includes all genera placed in Ixoreae, all genera classified in Pavetteae s.s., and several taxa from the remaining tribes of Ixoroideae s.l. The resulting phylogenies will also be used to assess the phylogenetic relationships between the Ixoreae genera.

P1269. Paraphyly of Paederieae, and recognition of Putorieae (Rubiaceae)M. Backlund¹, B. Bremer², M. Thulin¹;¹Evolutionary Biology Centre, Systematic Botany, Uppsala, Sweden,²Bergius Botanic Garden, Stockholm, Sweden.

The tribe Paederieae (Rubiaceae), until recently comprising 15 genera, has been regarded as paraphyletic in recent studies. To address this issue a molecular data set of *rbcl*, *rps16*, and *trnT-F* nucleotide sequences has been retrieved and analysed. The result unambiguously corroborates the suggestion that the Paederieae indeed is paraphyletic, and a grouping into two distinct clades is highly supported. One of these clades, Paederieae s.str., comprises the type genus *Paederia* together with the East Asian genera *Spermadictyon*, *Serissa*, and *Leptodermis*. Furthermore, *Paederia* divides into one Asian and one African group.

The genera *Putoria*, *Gaillonia*, (and its segregates), *Plocama*, and *Aitchisonia* form the other clade. This group has *pro parte* been recognized earlier, under the available name Putorieae. The sister group of the Putorieae are the tribes Theligoneae and Rubieae, the latter including *Kelloggia*, earlier placed in Paederieae. Some of the clades within Putorieae have a strong support from the molecular data, but they do not always correspond to the previously recognized genera and taxonomic changes seem inevitable.

P1270. Evolutionary history of the plant genus Pagamea Aubl. (Rubiaceae) in tropical South America - molecular markers, morphology and geographical variation.A. Vicentini^{1,2};¹University of Missouri Saint Louis, Saint Louis, MO, United States,²Missouri Botanical Garden, Saint Louis, MO, United States.

Pagamea comprises ca. 50 species (10 undescribed) of trees and shrubs that occur in forests and scrublands of nutrient-poor soils (white sands) throughout tropical South America. Pagamea is sister to Gaertnera, whose phylogenetic history indicates a recent rapid radiation throughout the Paleotropics. The molecular phylogenies of chloroplast (*rps16_intron*; *rpl20-5â€™rps12*) and nuclear (NOT, PER42) markers are contrasted with patterns of morphological variation and the geographical distribution of species. Phylogenetic results indicate a rapid diversification for Pagamea, though an older history than that of Gaertnera. These also indicate a dynamic biogeographical history, and incongruence between nuclear and chloroplast data suggest hybridization. Morphological clusters conform to monophyletic species for the most part, but there is also misleading morphological convergence. Dioecy has evolved multiple times and variation in breeding system occurs even at the population level. Results highlight the importance of combining molecules, morphology and geography for understanding species-level patterns and processes.

P1271. The Malagasy representatives of *Ixora* (Rubiaceae)

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Ixora (Ixoreae - Rubiaceae) is a large genus (300-400 species) of shrubs and small trees from humid forests. The genus is pantropical but species diversity is highest in Asia and Oceania. No world monograph of *Ixora* exists but recent publications include geographically restricted revisions, e.g. of the continental African species.

A revision of the Malagasy species of *Ixora* is underway. The genus is represented in Madagascar by c. 30 species, all endemic and many as yet undescribed. This number is slightly lower than that for the African mainland, but morphological variation is much greater. The following trends are found in the Malagasy species and absent on the continent:

- solitary flowers
- flowers up to 20 cm long (versus < 10 cm long in Africa). No observations are available but pollination of these long narrow flowers must be effected by hawkmoths.
- four-locular ovaries versus bilocular ones. This character is correlated with very large ovaries and calyces.
- large fruits

Once the revision is finished and species delimitations clear, the path is free to learn more about the affinities of the Malagasy species of *Ixora* and understand their radiation on the island.

P1272. Phylogeny and breakdown of distyly correlated with polyploidization in *Damnacanthus* (Rubiaceae).

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Damnacanthus is a small genus, is comprised of eight species, and is distributed mainly in laurel forests of the Sino-Japanese Region. Some species of *Damnacanthus* are distylous, and others have flowers with long styles and short stamens with few exceptions. In *Damnacanthus*, diploid ($2n = 22$) and tetraploid ($2n = 44$) counts were obtained by somatic chromosome observations. A strong correlation exists between chromosome number and occurrence of distyly. Regardless of taxa in *Damnacanthus*, distylous populations are diploid, and monomorphic populations are tetraploid. Molecular phylogenetic analysis using some non-coding chloroplast DNA regions suggests multiple origins of polyploidization in *Damnacanthus*. Tetraploids appear in different lineages with diploids in the present molecular phylogenetic tree. Thus, polyploidization seems to have caused the breakdown of distylous to monomorphic flowers.

P1273. Lectotypification of *Callicocca ipecacuanha* Brot. and neotypification of *Cephaelis acuminata* H.Karst., with reference to the drug ipecac

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The main pharmacopoeias cite the roots of *Cephaelis ipecacuanha* (syn. *Callicocca ipecacuanha* Brot.) together with the roots of *Cephaelis acuminata* H.Karst. as the sources of the crude drug ipecac (European Pharmacopoeia, 2002, 2004; United States Pharmacopoeia, 2004; British Pharmacopoeia, 2003; Japanese Pharmacopoeia, 2001). Ipecac is an important emetic and expectorant used in case of poisoning, mainly in children. However, *C. acuminata* does not occur in botanical literature, except in connection with the original description, and this falls entirely within the variation of the widespread and variable *Cephaelis ipecacuanha*. *Callicocca ipecacuanha* was described from Brazil, but no type specimen has been found and the name is here lectotypified with an illustration from the protologue. For *Cephaelis acuminata*, described from Colombia, no original material is extant, and a recent specimen from Colombia is here selected to serve as neotype.

P1274. The molecular approach of different taxonomical levels in Rubiaceae

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The aim of this study was: 1. Separating three genera of *Rondeletia* complex; 2. The justification of the species level in the *Hintonia* genus; 3. The better cognition of interior separation of *Randia* genus.

A molecular phylogeny was constructed using nucleotide sequences of internal transcribed spacer (ITS) regions of nrDNA

and of the rps16 intron of the cpDNA. A phylogenetic tree was constructed from the nucleotide sequence alignment using PAUP* 4.0.

The rps-16 intron seems to be useful to identify different genera. By analyzing this sequence, *Arachnothryx*, *Calicosae*, *Rogiera* genera were well separated within *Rondeletia* complex, *Rondeletieae* tribe; *Hintonia* genus within *Cinchoneae* tribe; *Randia* genus within *Gardenieae* tribe

With the analysis of ITS sequence, significant polymorphism manifested at specific level in case of all the studied species. This shows the sequence's possible utility in species' identifying.

During the study of both sequences we found two, well separated groups between *Randia* species selected for this research. After this result we found also morphological characteristics, which differentiate the two groups above.

P1275. Grapevine (*Vitis* spp. L.) Old Varieties and Land Races Conservation in Slovakia

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The part of the National program of plant genetic resources for food and agriculture is also conservation and sustainable use of old varieties and land races of grapevine. Therefore the monitoring, inventory control, reproduction and long term conservation of genotypes is provided. In the process of evaluation were analyzed 35 quantitative and qualitative characters for a leaf, bunches, a berry and seeds. For these characters has been determined following variability ranges - the length of a bunch stalk (30.67 - 43.87 mm), the weight of a single bunch (71.4 - 200 g), the length of a bunch (88.20 - 181.67 mm), the width of a bunch (63.73 - 111.93 mm), the number of berries per bunch (35 - 178 no), the number of seeds per 100 berries (130 - 315 mm no), the weight of 100 dried seeds (1.67 - 6.45 g). Duplicated genotypes are preserved in the clonally repository. The important genotypes are reproduced for practical use. Experimental data and image documentation are prepared for information system GENOTYPDATA *Vitis*.

P1276. Wild grapevine (*Vitis vinifera* ssp. *silvestris*) in Europe: Distribution, ecology, growth strategy and threats

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Lausanne, Switzerland.

Wild grapevine (*Vitis vinifera* ssp. *silvestris*) used to be widely distributed across Europe, but today its populations are fragmented and reduced to less than one hundred individuals. Morphologically and genetically, this crop-related species can be clearly differentiated from cultivars. It is adapted to many different environments in Europe such as floodplains or colluvial forests, but at the local scale is found in similar environments (of vegetation and soil). The largest populations are located in Austria where the floodplain dynamic and forest management still allow a sexual reproduction. Silviculture has a direct impact on the disappearance of wild grapevine, while river management, the introduction of pests, diseases and American species have a greater yet indirect impact. Specific examples of European populations as well as measures of conservation will be given.

P1277. Wild grape biodiversity detected by DNA molecular markers

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The *Vitis vinifera* L. species includes: *V. vinifera* ssp. *silvestris*, the wild subspecies, distributed in the spontaneous vegetation in West-Asia, Mediterranean basin and Central and Southern Europe, and *V. vinifera* ssp. *vinifera*, related to cultivated varieties including about 8000 accessions. Wild grape is dioecious species grows in humid soils, like river banks, where it is leaned to the arboreal vegetation. In recent years, the maintenance of genetic variability within wild grape populations is at risk due to decline of

wetland areas, with the conversion of land to agriculture. To preserve genetic diversity of this species, we analysed the genetic variability in several wild grape populations collected from different European and Middle East regions, by using SSR markers. Results provide information about population genetic structure and gene flow among different grape populations and identify areas harboring high levels of wild grape variability. These data are able to define the suitable conservation strategy.

P1278. Phylogeography of *Viola Persicifolia*

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Viola persicifolia (Violaceae) is a wide spread but rare plant species occurring throughout Europe with the exception of the Mediterranean and arctic Europe. It is paleotetraploid and the parental species of several closely related allopolyploids (*V. canina*, *V. lactea*, and *V. pumila*). Two infraspecific taxa have been described: *V. persicifolia* var. *persicifolia* and var. *lacteaoides*. The latter one is only known from The Netherlands and might be one of its few endemics. We are studying the phylogeography of *V. persicifolia* in order to gain insight into its dispersal routes across Europe and its arrival in The Netherlands. We hope to find out (1) whether the distribution area of *V. var. lacteaoides* is restricted to The Netherlands, and (2) whether *V. var. lacteaoides* is either a relict population of a larger distribution area that existed in the past, or a local genotype that originated *in situ* in the valley of the IJssel river. In this presentation, results of a preliminary study using a chloroplast marker will be shown.

P1279. Origin of the Parma Violet cultivars (Violaceae)

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In order to clarify the relationships among Parma Violet cultivars and wild species we conducted a phylogenetic study based on ITS and allozymes markers. These cultivars are characterized by fragrant abnormal flowers with 20-40 floral pieces and stoloniferous habit. Based on ITS data, Parma Violets are nested in an unresolved *Viola alba* and *V. sintenisii* clade, and two groups of cultivars can be identified. One of these two cultivar groups is characterized by its highly polymorphic ITS sequences that may be related to vegetative propagation. The remaining group had homogeneous ITS sequences and may result from cuttings from rare seedlings. With allozymes, the cultivars showed strong relationships to either of two species, based on allozymic composition and the presence of shared private markers. Four private markers connected the Parma Violets to the variable *V. alba* complex, one to the West Asian *V. sintenisii*, and one to both *V. alba* and *V. sintenisii*. These results imply an assignment to *V. Alba* for the Parma Violet cultivars, most probably *Viola alba* ssp. *dehnhardtii*. They, most likely originated once and loss of heterozygosity is due to rare events of sexuality.

P1280. How much we know about violets from Argentina?

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The cosmopolitan *Viola* L. genus is represented in Argentina by near 50 species, mainly native ones and only four adventitious entities. Some species, the "yellow violets" from section *Chilenium*, are one of the most common components of the Patagonia and Tierra del Fuego rain-forests. Other taxa belonging to the very interesting section *Andinium* occur in Argentine northwestern, at high altitudes, mostly between 3000-4000 m above sea level. We know very little about these last species, most of them, endemic, confined to small geographical areas and difficult to be collected. In order to assess utility of different morphological, anatomical and cytological characters for the classification, we present preliminary data gathered from a screening study taking in account some representative species. We also provide data concerning species distribution.

Selected characters as chromosome numbers and pollen morphology observed at SEM, are discussed. Leaf and seed anatomical features are highlighted, showing the results their potential value in taxonomic and evolutive studies.

P1281. *Corynostylis* a true violaceous neotropical genus.

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Violaceae is a medium sized cosmopolitan family, that grows in almost all terrestrial environments with amazing morphological plasticity. More than 50 % of the species belong to the putatively derived tribe *Violeae* (worldwide) and share the commonest characters that distinguish the violets. *Corynostylis* is a typical violaceous neotropical genus with 4 shrubby or lianiform species, it possess alternate serrulated leaves, deciduous stipules, strongly zygomorphic, resupinated entomophilous flowers, quincuncial sepals aestivation, apotact petals, anterior petal wider and long spurred, free stamens, calcarate and villous dorsal glands, laminar connective scales, tricarpellate ovary, curved style, 3 valvate woody capsule, numerous seeds compressed and sub square. Species and their distribution are: *C. arborea* (Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guyana, Mexico, Nicaragua, Panama, Peru, Surinam, Trinidad (introd), Venezuela), *C. carthagenensis* (Colombia, Venezuela), *C. pubescens* (Bolivia, Brazil, Venezuela) and *C. volubilis* (Brazil, Colombia, Peru, Venezuela) with a possibly new from Venezuelan Amazonas.

P1282. The Digitalization of Rare Wall Chart Collections (the largest digitalized collection in Europe)

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Our department owns valuable complete and also non-complete collection of pictures on general and systematic botany - *Botanische Wandtafeln* (published from 1874 to 1914, Berlin, Paul Parey), *Pflanzenphysiologische Wandtafeln von Frank und Tschirch* (published from 1889 to 1894, Berlin, Paul Parey), *Botanical Atlas by Arnold & Carolina Dodel-Port* (published from 1878 to 1883, Switzerland) etc. Most of these pictures have an invaluable worth. All these wall pictures (about 600 pieces) have been digitalized. The digitalization enables the students to understand complicated morphological-anatomical structures of plants as well as to recognize characteristic signs of representatives of plant families. Every picture was retouched and supplemented by scientific commentaries. The picture database is placed on the web pages of the department. The studies were financed from grants of Higher Education Development Fund 1097/2002, 816/2004.

P1283. The life and scientific achievements of Emil Godlewski Snr (1847-1930) - a Polish plant physiologist

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Emil Godlewski Snr (1847-1930) was a Polish scientist, who contributed to the development of plant physiology worldwide, as a modern and separate branch of botanical sciences which emerged at the mid-19th century. He authored many publications, some of them are considered today to be 'classics'. He carried out his first experiments in plant physiology during a half-year stay at the well-known laboratory of Professor Julius Sachs in Würzburg, Germany (1872). From that time on, he devoted all his time to this discipline, working in Polish universities and other higher education institutions in Cracow, Lvov, Dublany (near Lvov) and Pulawy. He earned also high recognition as educator and organizer of scientific activities.

E. Godlewski worked on several topics in the field of plant physiology, including: photosynthesis, respiration, growth, etiolation, metabolic transformations of protein, water transport, nitrification and mineral nutrition. He also published works on agrochemistry. The merit of his achievements can be also measured by the fact that numerous academies of sciences and learned societies in Poland and abroad made him their member.

P1284. E. Janczewski's materials on Ribes taxonomy at the Jagiellonian University.**K. Kulpinski;**

Botanical Garden, Jagiellonian University, Krakow, Poland.

Edward Janczewski (1846-1918) was a plant anatomist and taxonomist, professor of the Jagiellonian University (Krakow). His "Monographie des Groseilliers" on genus *Ribes* was the first wide taxonomical analysis of this genus. Published almost a century ago (1907), it retained most of its value as the best source of information on this genus, regardless of the fact that only half of the world species were described. The Institute of Botany at the Jagiellonian University owns a vast collection of materials Janczewski used during his work on the monograph. It includes herbarium, microscope preparations and some living plants cultivated at the University's Botanical Garden. Some of those materials were used by Janczewski to describe new taxa. The main aim of the first part of my project is to create digital database of all materials, then analyse them regarding their importance. The results of this part will be then used in the next phase, in which I will try to evaluate the real influence Janczewski had on *Ribes* taxonomy, and also on plant taxonomy as a science. I will also try to describe current state of *Ribes* taxonomy and summarize changes in it since Janczewski's monograph.

P1285. The element nitrogen in the philatelic world**H. Bothe;**

Botanisches Institut, Universität Köln, Köln, Germany.

Stamps issued world-wide provide an excellent opportunity to display diverse aspects of the element nitrogen. Copies of stamps issued over the years in the world will be shown on this poster with four spaces. The poster describes the discovery of the element nitrogen from the beginning on, the occurrence of the element nitrogen in selected organic molecules as well as the impact of nitrogen on pollution both in the soil and on the atmosphere. In addition, processes like dinitrogen fixation or denitrification will be demonstrated on stamps. Diverse plants (legumes and others) which live in symbiosis with dinitrogen fixing bacteria (*Rhizobium*, *Frankia*) will be shown. Examples of carnivores will also be displayed. The copy of each stamp displayed is supplemented with a text explaining the relevance of the stamp with respect to the element nitrogen. The presentation will also be available on CD.

P1286. The original Willdenow's collection in Herbarium of Saint-Petersburg University (LECB)**V. A. Bubyreva¹, V. V. Byalt², L. V. Orlova², A. V. Zhuk¹;**¹St.Petersburg State University, St. Petersburg, Russian Federation,²Komarov Botanical Institute RAS, St. Petersburg, Russian Federation.

The Herbarium of Saint-Petersburg State University (LECB) is one of the oldest Russian Herbarium and includes about 850000 specimens. Several years ago in the separate A.G.H. Bongard's collection we have found herbarium specimens with handwriting of C.L. Willdenow (it was confirmed by P. Hiepko from Berlin). This fact is not known for most of scientists nor Russia nor world wise. In 2004 under support of the Presidium Russian Academy of Sciences grant "The visualisation of J. Boeber and C.L. Willdenow's specimens preserved at St. Petersburg university (LECB)" we have looked through the whole Bongard's collection (ca. 250000 specimens) and found 1088 original Willdenow's specimens of 54 families of the Higher plants. It is possibly the specimens were received by Bongard not directly from Willdenow as Bongard was young scientist when the first was yet alive. Preliminary study of specimens have shown that near 100 authentic Willdenow's specimens of 23 families preserved in LECB and several names were not included in "Herbarium Willdenow Alphabetical Index", 1974. Thank to this fact importance of LECB collection strong increased

P1287. Phylogenetic study of *Antrodia* and other related genera based on analysis of LSU and SSU nuclear rDNA**S. Wu¹, Z. Yu², C. Chen¹;**¹National Museum of Natural Science, Taichung, Taiwan Republic of China, ²School of Life Science, Yangtze University, Hubei, China.

Antrodia is polypore genus with almost 40 species, causing a brown rot in wood. This study aims in evaluating generic status of *Antrodia*, as well as its phylogenetic relationships with other polypore genera with more or less similar characters. Phylogenetic

analysis of this study was based on sequence data from LSU and SSU nuclear rDNA. All studied *Antrodia* species form a poorly supported lineage (*Antrodia* s.l.), which also accommodate species of some other genera. Three monophyletic groups could be identified in this lineage. The group of *Antrodia* s.s. accommodates many species of *Antrodia*, and *Fomitopsis* and *Piptoporus*. The second group represents the genus *Amyloporia*, characterized in having bitter taste of fruiting body and amyloid skeletal hyphae. The third group represents the genus *Fibroporia*, characterized in having fruiting body with rhizomorphic margin. Independence of a recently proposed genus, *Taiwanofungus*, was supported in this analysis. *Antrodiella* and *Diplomitoporus* are two white-rot genera resembling *Antrodia* by sharing general morphological features. The result indicates that these two genera are not closely related to *Antrodia*.

P1288. Three taxonomic forms of *Dictyostelium purpureum* from Taiwan and molecular phylogeny of dictyostelids in East Asia**Z. Y. Yeh, U. F. Wang, Y. P. Tan;**

National Taiwan Normal University, Taipei, Taiwan Republic of China.

Twelve isolates of *Dictyostelium purpureum* were obtained from various sites of forest soils in Taiwan. Among them, three taxonomic forms of *D. purpureum* were discovered based upon morphological characteristics. One was "temperate form", the second was "subtropical form" and the third form was newly found and was named "medial form". Molecular phylogenetic analysis was performed also in this study. Nuclear ribosomal DNA- ITS2 sequences of the three forms of *D. purpureum* and another 27 dictyostelid isolates from Taiwan and East Asia were analyzed and phylogenetic tree was constructed by PAUP program. Three forms of *D. purpureum* can be clustered into 3 groups. The tested 39 isolates of *Dictyostelium* are split into two well-supported clades based on polar granule present isolates and polar granule absent isolates. Polar granule is one of the most important characters and may be considered to serve as a taxonomic criterion on the subgenus rank under *Dictyostelium*.

P1289. Biodiversity studies of Leotiales in Taiwan**M. L. Wu;**

Graduate School of Environment Education, Taipei Municipal Teachers College, Taipei, Taiwan Republic of China.

Leotiales is a very important family of discomycetes in the resources of bioactive metabolites. More than 2,000 species of Leotiales are currently known. However, regional mycoflora were mostly investigated in Europe and North America but not southern part of the world including Taiwan. Studies of the Leotiales in Taiwan during 1919–2004 are briefly reviewed. There were 54 species of Leotiales in 6 families and 24 genus had been reported from Taiwan. They may be from the area of general deciduous or mixed deciduous and coniferous forests to the area with high acidity soil weathered in warm volcano mist. The habitats and hosts of the known species have been analyzed based on published information. In order to glance at progress on studies of Leotiales in Taiwan, a list of reported species, genus and the researchers with publications related to this kind of fungi are provided. The ecological and morphological characteristics of the Leotiales reported in Taiwan as well as 4 newly recorded species will be discussed.

P1290. Taxonomic Studies on Species of *Russula* in Taiwan**E. F. T. Tschén;**

National Museum of Natural Science, Taichung City, Taiwan Republic of China.

Russula (Russulaceae) is one of the major mycorrhizal macrofungal genera that occur in broad-leaved, mixed and conifer forests in Taiwan. A taxonomic study of *Russula* (Russulaceae) has been undertaken in Taiwan during 2000-2002, based on specimens collected at 500-2,500 m altitude. A total number of 17 species were obtained from this study. They are: Subgenus *Compactae*: *Russula nigricans*, *R. delica*. Subgenus *Russula*: *R. cyanoxantha*, *R. virescens*, *R. amoena*, *R. violeipes*, *R. alboareolata* (Sect. *Heterophyllae*), *R. castanopsidis* (Sect. *Pachycystides*), *R. foetens*, *R. subfoetens*, *R. laurocerasi*, *R. senecis* (Sect. *Ingratae*), *R. emetica*, *R. betularum*, *R. sanguinea*, *R. fragilis* (Sect. *Piperinae*), *R. xempelina* (Sect. *Polychromae*). *R. violeipes*, *R. subfoetens*, *R. betularum* and *R. xempelina* are reported for the first time in Taiwan. *R. alboareolata*, *R.*

castanopsidis and *R. senecis* are E Asia species, only distributed in Japan, Korea, China and Taiwan.

P1291. Diversity and Molecular Phylogeny of ballistoconidium-forming yeasts from China

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From the ballistoconidium-forming and related basidiomycetous yeast strains isolated from plant material collected in representative regions of China, more than 90 species belonging to 14 genera have been identified based on phenotypic and molecular characterization. Approximately one third of the species identified were new to science. The high frequency of new species isolation indicates that the species diversity of basidiomycetous yeasts in nature is very high and that a considerable number of new taxa remain to be found.

The phylogenetic relationships of the new basidiomycetous yeast species from China with described species were revealed from 18S rDNA, ITS region and 26S rDNA D1/D2 domain sequence analyses. Several new distinct clades in different lineages of basidiomycetous yeasts have been recognized by the addition of new species to existing phylogenetic trees. The new clades are significant to reclassify the basidiomycetous yeast species into more homogeneous groups.

P1292. *Verticillium* is still polyphyletic

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²Plant Pests & Diseases Research Institute, Tehran, Islamic Republic of Iran.

Section *Prostrata*, was treated by Zare and Gams (*Nov. Hedw.*, 2000-01). Three new genera, *Lecanicillium*, *Simplicillium* and *Haptocillium* were introduced. Section *Verticillium* is not closely related to sect. *Nigrescentia* and, after the proposed conservation of *Verticillium* with *V. dahliae* as conserved type (Gams *et al.*, *Taxon*, 2005), this species is now reclassified in *Acrostalagmus* as *A. luteo-albus* (Link : Fr.) Zare, W. Gams & Schroers (*Zare et al. Mycol. Res.*, 2004). Section *Nigrescentia* comprises the important plant-pathogenic species *V. dahliae* and *V. albo-atrum* plus a few other weaker plant pathogens. In this project, section *Albo-erecta* with erect conidiophores and mainly fungicolous species around *V. fungicola* is being investigated using classical and molecular techniques. Some 140 isolates from all over the world are examined. The results show an extensive variation among isolates examined; the observed relationships correlate with the ecology of the isolates, suggesting the need for further generic segregation. At least three new genera will have to be introduced, but no new anamorph-teleomorph connection is yet found.

P1293. Preliminary study on relationships among genera in the Pyrenomataceae (Ascomycetes, Fungi)

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The Pyrenomataceae contains a large group of operculate discomycetes. Five subfamilies and many tribes have been designated based on morphological features. To investigate the relationships among genera of the family, 18S nrDNA partial sequences of 24 genera were analyzed and a neighbor-joining tree was obtained with *Saccharomyces cerevisiae* as outgroup. Our results indicated that 3 major clades are recognized. One represented by taxa of *Acervus*. The second clade consists of 2 sister groups, *Otidea* and *Otideopsis* as one together with but obviously not close-related to *Lamprospora*, *Leucoscypha*, *Neottiella*, *Octospora* and *Rhodoscypha* sharing similar anatomic structures and inhabitation. The largest clade contains rest of the genera tested, in which 2 groups are distinguishable with only 33% bootstrap support. One is of *Scutellinia*, *Aleuria*, *Melastiza*, *Humaria*, *Trichophaea*, *Trichophaeopsis*, *Jafnea*, *Sowerbyella* and *Cheilymenia* in which hyphoid hairs or setae exist on receptacle surface; and the other consists of *Geopyxis*, *Paurocotylis*, *Tarzetta*, *Pulvinula* and *Chalazion*. The family, as currently defined, might not be monophyletic.

P1294. A white form of *Auricularia fuscusuccinea* from Lacandonia tropical forest, Chiapas, Mexico.

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Auricularia species have tough to rubbery gelatinous and auriform (from Latin auriculae = ear) basidiomes. All are saprotrophic and grow in trunks of trees (alive or dead). Cultivation has been developed mainly in Asian countries. Records on edibility back from 200-300 B.C. in China. In the Dictionary of Fungi (Kirk *et al.*, 2002) are considered approximately 15 species worldwide and the Index of Fungi listed 124 names published. Kobayasi (1981) described 15 species and 5 forms. From the Neotropics seven species are reported (Lowy, 1971). Species delimitation has been based on the internal structure of basidiome (Lowy, 1952).

A white form of *Auricularia fuscusuccinea* is described for the first time from the Tropical Forest in the state of Chiapas, Mexico. White forms have been described so far only for *A. auricula* f. *albicans* (*Hirneola auricula-judae* var. *lactea*), *A. polytricha* f. *leucochroma* and *A. delicata* f. *alba*. The main characters of this form are basidiome colour, abhymenial hairs length, context internal structure and size of basidia and spores. It was found growing on unidentified trunk in Lacanjá-Chansayab, Ocosingo Municipality.

P1295. Molecular evidence for the taxonomic status of *Lycopersicon* within sect. *Petota*.

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Members of the genus *Solanum* L., the largest of the genera of the family Solanaceae, have been frequently used as an important model system for studies on structural and functional genomics. Hence, clear understanding of phylogenetic relationships and evolutionary trends within the genus is necessary for appropriate interpretation of the genomics data and to enlarge the efficiency of breeding programs involving wild genetic material. To clarify the taxonomic status of tomatoes (*Lycopersicon* ssp.) and their relationship to other members of *Solanum*, in particular to potatoes (sect. *Petota*), the organization of the rDNA external transcribed spacer (5' ETS) was studied in 35 wild and cultivated *Solanum/Lycopersicon* species. Sequence analysis showed that evolution of the 5' ETS within the group proceeded mainly by amplification/duplication of structural elements. Phylogeny reconstruction confirmed that tomatoes definitely belong to the sect. *Petota* having a common ancestor with Central American/South American primitive potatoes.

P1296. Introgression and Paralogy of ETS in Myrtaceae?

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Sequence data from the external transcribed spacer (ETS) region within the tribe Chamelaucieae provides a highly resolved phylogeny more congruent with morphology than that based on chloroplast data. However, numerous instances of intraspecific polymorphism occur indicating incomplete gene conversion. Polymorphisms are usually single base substitutions but indels and multiple polymorphisms also occur. Intraspecific variants have not been recorded in the disparate genera *Metrosideros*, *Syzygium* and *Kunzea* from other tribes in the family, although there is a possibility that they may occur in *Eugenia*. The phenomenon is not uniform across the tribe - some taxa show no variation indicating that nrDNA arrays within individuals are homogeneous. Where variation occurs, copy number may be unequal; this could be a PCR artefact but may reflect the ongoing process of homogenization. Polymorphisms are usually random but some closely related taxa share multiple polymorphic sites, indicating relatively recent introgression.

P1297. Non-concerted ITS evolution in the genus *Mammillaria* (Cactaceae)

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The ITS region: ITS1+5.8S rRNA+ITS2 of the ribosomal DNA belongs to a multiple gene family with hundreds of tandemly

repeated copies. Thus, it should be subjected to concerted evolution.

Our research deals with 21 out of over 300 *Mammillaria* species representing plants from all subgenera (except *Cochemiae*) as well as the sections and series of the large subgenus *Mammillaria*.

The molecular study revealed several different copies of the ITS region (sequence, length) on individual level. The main differences were observed within the 5.8S rRNA genes. Some of these belong to apparently functional genes whereas others are probably non-functional. The constructed Neighbour-joining tree shows, that ITS regions of different *Mammillaria* species containing putative 5.8S rRNA genes cluster within the tree, whereas pseudogenes are distributed all over.

Our result of non-concerted ITS spacer evolution in *Mammillaria* is against the traditional assumption of concerted evolution.

P1298. Subnucleolar localization of rDNA and NopA100 are affected by clinorotation

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Plants are the main object in investigations of gravity role in life on Earth. Now this research has taken on special significance when long-term expeditions to the Moon and Mars are planned. Plant cell nucleolus is known to reflect the level of cell metabolism and to monitor the influence of environmental stimuli on a cell. We studied root meristematic cell nucleoli of two-day *Pisum sativum* seedlings grown in the control and under clinorotation with immunogold electron microscopy. In the control, the labeling with anti-DNA and anti-NopA100 antibodies was revealed in a nucleolus on FCs and DFC. FCs were considerably more densely labeled than DFC. Under clinorotation, we showed a redistribution of rDNA and NopA100 between FCs and DFC in a nucleolus as compared to the control. FCs and DFC were much more labeled with an anti-DNA antibody and contained significantly less quantity of NopA100 than in the control. The alterations in localization of rDNA and NopA100 in the nucleolar subcomponents testify about a decrease in the level of rDNA transcription and processing of rRNA allowing us to propose lowering the functional activity of a nucleolus influenced by clinorotation.

P1299. Phylogeny and evolution of flower characters in European *Heracleum* L. (Apiaceae: Apioideae)

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The genus *Heracleum* consists of approx. 80 species distributed mainly in Asia and Europe. The paper analyses all European taxa for their phylogenetic relationships and evolution of morphological characters associated with reproduction. Phylogenetic trees inferred from ITS rDNA sequences show that all European taxa fall within *Heracleum* clade (as opposed to Asiatic *H. rigens*, which is closer related to *Zosima orientalis*). All members of *H. sphondylium* complex, as described in Flora Europaea, cluster together, which is a result of identical ITS sequences. The same situation is found in *H. mantegazzianum* and *H. pubescens* branch. Several mountain species (*H. minimum*, *H. carpaticum*, *H. ligusticifolium*, and *H. austriacum*) form a distinct clade supported by high bootstrap value. Most of the 46 studied morphological characters were homoplastic, except flower color and flower symmetry. The analyses suggest that taxa with greenish actinomorphic flowers (eg *H. sphondylium* subsp. *sibiricum*) are evolutionary advanced contrary to ancestral taxa with white zygomorphic flowers.

P1300. Systematic Studies of *Vallisneria* L. in South Korea

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The morphological and molecular systematic studies of *Vallisneria* L. (Hydrocharitaceae) in South Korea were conducted to identify infraspecific taxa showing variation of seeds. Total 221 individuals of *V. asiatica* Miki and *V. denseserrulata* Makino were collected from 22 different regions of Korea, and 61 morphological characters were examined for the principal component analysis (PCA) and unweighted pair group method with arithmetic mean (UPGMA) analysis. The internal transcribed spacer (ITS) region of the 18S-25S nuclear ribosomal DNA repeat was sequenced from

25 individuals (11 males and 14 females) of *Vallisneria* in Korea, and maximum parsimony trees were generated from the ITS sequences including those of *V. americana*, *V. nana*, *Nechamandra alternifolia* and *Hydrilla verticillata* as outgroups. As the results of our studies, *V. asiatica* Miki var. *protuberans* Na et Choi is characterized as a new variety and *V. denseserrulata* Makino var. *spinulosa* (Yan) Na et Choi is recognized as to need the new combination.

P1301. Photosystem II gene sequences of psbB and psbC clarify the phylogenetic position of Vanilla (Vanilloideae, Orchidaceae)

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Nucleotide sequences of the plastid genes psbB and psbC were obtained for 34 taxa representing nearly all genera currently classified within Vanilloideae (Orchidaceae). These genes code for two proteins that make up the Photosystem II and have only rarely been used for reconstructing phylogenetic relationships among plants. Nucleotide variation within each of these genes is sufficient to resolve the major relationships among most Vanilloideae and the combined two-gene tree is fully resolved and highly supported at the genus level. These gene trees demonstrate with a high degree of confidence (95% jackknife support) that a clade of mostly achlorophyllous tropical vines including *Pseudovanilla* and *Erythrorchis* are the sister group to *Vanilla*. The two New Caledonian endemic genera of Vanilloideae are sister to this pair, and *Epistephium* is sister to all Vanilloideae. With this new interpretation of vanilloid orchid relationships in hand, it is possible to re-examine the evolutionary pattern of such characters as winged seeds, unilocular ovaries, and climbing habit within Orchidaceae.

P1302. Molecular systematics and biogeographic patterns in Thunbergioideae s.l. (Acanthaceae) based on phylogenetic analyses of nuclear and chloroplast sequence data

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Thunbergioideae s.l. formerly were divided into two subfamilies, the Thunbergioideae and the Mendoncioideae, or even were considered to constitute two separate families. Recent morphological and molecular studies support the reduction of Thunbergioideae and Mendoncioideae to a single subfamily within Acanthaceae. However, relationships within Thunbergioideae s.l. have been little studied and are still poorly understood. The family consists of five genera. The largest of which, the genus *Thunbergia*, contains about 100 species restricted to tropical and subtropical regions of Africa and Asia. *Mendoncia*, the second largest genus, contains around 60 species and is most diverse in Central and South America, but also includes a few African representatives. The main goals of the present study are to test 1) whether earlier, morphology-based subgeneric divisions of *Thunbergia* are congruent with molecular evidence; 2) whether Asian *Thunbergia* species form a monophyletic group; 3) whether New World and Old World *Mendoncia* species are reciprocally monophyletic; and 4) to answer the question about the likely geographical origin of the group as a whole.

P1303. A phylogenetic study of water-hemlock (*Cicuta*; Apiaceae tribe Oenantheae)

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The genus *Cicuta* is the most virulently poisonous group of flowering plants native to the north temperate zone. The most recent treatment recognized four species (*C. maculata*, *C. douglasii*, *C. bulbifera*, and *C. virosa*), with *C. maculata* divided into four varieties. The evolutionary relationships among these taxa, and the characters best to distinguish them, are unclear. We present the results of a phylogenetic analysis of morphological characters and DNA sequences from the nuclear rDNA ITS region. Data from the chloroplast 5' exon trnK-rps16-trnQ-psbK-psbI region are currently being obtained. Preliminary results suggest that the

North American *C. bulbifera* and the circumboreal *C. virosa* are each monophyletic, the latter species sister to all other taxa. *Cicuta douglasii* is highly polyphyletic, with all examined accessions but one arising within *C. maculata*. This single accession is from central California and is successively basal to *C. virosa* in the cpDNA-derived trees. Within *C. maculata*, only western North American var. *angustifolia* is retained as monophyletic; all other taxa comprise a morphologically heterogeneous *C. maculata* var. *maculata*.

P1304. Molecular and morphological variation in *Myosotis laxa* Lehm. in the Baltic Sea region and the status of *M. laxa* ssp. *baltica*

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Two subspecies of *Myosotis laxa* Lehm. (Boraginaceae, genus *Myosotis* section *Palustres*): *M.l.* ssp. *baltica* (Sam.) Hyl ex Nordh. and *M.l.* ssp. *caespitosa* (Schultz) Hyl. ex Nordh., were studied using both morphological and molecular characteristics. rDNA ITS1 and ITS2 sequences, which have been proved to work in the section *Palustres*, were used. *M. l.* ssp. *baltica* is considered to be a microendemic taxon in the Baltic Sea region. In some publications it has been stated not to occur in the southern part of the Baltic Sea region (Estonia incl.). In the present work it was proved that 'typical' specimens belonging by morphological features to ssp. *baltica* are occurring in Estonia also. A continuous variation from ssp. *baltica* to ssp. *caespitosa* can be recorded here as well as it was described on the material originating mainly from Sweden. As the result of molecular analyses it occurred that morphologically typical specimens of two subspecies often do not differ genetically. The hypothesis of one center of origin is therefore not true. *M.l.* ssp. *baltica* is probably developing from an ecotype to a new microendemic taxon in several places separately.

P1305. A phylogenetic study of endemic Iranian Umbelliferae based on rDNA ITS sequences

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Taxonomic diversity of the Umbelliferae in Iran is one of the highest in Asia. Our present aim is to check taxonomic relationships in some Iranian Umbelliferae, especially in endemic genera, using the independent criterion of rDNA ITS-sequencing. As a result, 34 species from 26 genera were subject in molecular taxonomic analysis of the Umbelliferae for the first time. Phylogenies were constructed by neighbor-joining, parsimony analysis and Bayesian methods. Some results are unexpected. The analysis of resulting phylogenetic trees indicates that: (1) *Hausknechtia*, one of the most enigmatic genera among Iranian Umbelliferae, is the closest relative of *Demavendia* and *Zeravschania*; (2) new described monotypic genus *Mozaffariania* Pimenov & Maassoumi is the close relative of *Ferula-Leutea* clade; (3) *Ducrosia anethifolia* in all trees is situated in the basal position of enlarged *Heracleum*-clade; (4) *Calyptrosciadium*, a small Irano-Afghanian genus, is a new member of *Komarovia*-clade. In the cases of *Ducrosia*, *Mozaffariania*, *Hausknechtia*, *Demavendia* and *Calyptrosciadium* molecular data throw some light on the taxonomic affinity of genera studied.

P1306. Polyphyly of tribe Laserpitieae and its inclusion in tribe Scandiceae subtribe Daucineae (Apiaceae): inference from fruit morphology and rDNA ITS data

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Tribe Laserpitieae (family Apiaceae) is comprised of species that are characterized by dorsally compressed fruits with winged secondary (vallecular) ridges. Phylogenetic analyses using rDNA ITS sequence polymorphism data have shown that all member genera of Laserpitieae fall within tribe Scandiceae subtribe Daucineae. Both in MP and NJ trees, a majority of Laserpitieae form a grade in relation to the spiny-fruited members of Daucineae. Several others are nested within the paraphyletic

Daucus. The genus *Laserpitium* appears to be polyphyletic and includes at least four clades. Mapping of fruit characters on the phylogenetic tree suggests that winged fruits are plesiomorphic for Daucineae (with Laserpitieae included). Switches from anemochory (winged fruits) to zoochory (spiny fruits) and back to anemochory occurred several times on diverse branches of the tree. All analyzed characters from fruit morphology and anatomy appear to be homoplastic.

P1307. Polyphyly of the genus *Ferula* (Apiaceae) and the affinity of most of its members to tribe Scandiceae based on rDNA ITS sequence variation

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The genus *Ferula* is traditionally placed in tribe Peucedaneae. Recent molecular investigations using rDNA ITS sequence variation have revealed that the genus is polyphyletic with its type, *F. communis*, and two other species, *F. assa-foetida* and *F. tingitana*, placed in the apoid superclade while the other species formed a clade within tribe Scandiceae. We sequenced the rDNA ITS region from 50 other accessions of *Ferula* and performed phylogenetic analyses using MP and NJ. In the trees obtained from those analyses, all newly sequenced taxa have arisen within tribe Scandiceae. These species formed a well-supported clade. However, the affinities within this clade remain unresolved. This clade formed a polytomy with other lineages of Scandiceae corresponding to subtribes Scandicinae, Daucinae, and Torilidinae. Another addition to Scandicinae turned out to be *Glaucosciadium cordifolium*. In these trees, it constituted a separate lineage with no immediate relatives.

P1308. Phylogenetic relationships within *Chaerophyllum* (Apiaceae) as inferred from nuclear rDNA ITS sequence variation

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The phylogenetic relationships among the members of the genus *Chaerophyllum* (tribe Scandiceae, family Apiaceae) were estimated from the nucleotide sequence variation in the ITS1-5.8S-ITS2 rDNA region using maximum parsimony, neighbour-joining and maximum likelihood methods. Thirty-eight species were sampled. Those with a broad geographic distribution were represented by several accessions in the analyses. Three major clades were inferred corresponding to the recently redefined or established sections: *Chaerophyllum*, *Dasyptalon*, and *Chrysocarpum*. The monotypic sect. *Physocaulis* appeared to be sister to sect. *Chaerophyllum*. With the exception of *C. heldreichii*, all newly sequenced taxa occurred in section *Chrysocarpum* with the Himalayan *C. villosum* placed sister to the remaining members of this section. *Chaerophyllum prescottii*, often regarded as a subspecies of *C. bulbosum*, appears to be a distant relative of the latter. A new addition to sect. *Chaerophyllum* is *C. heldreichii*, endemic to Greece.

P1309. Phylogeny and biogeography of the Pacific genus *Meryta* (Araliaceae) based on ITS and ETS sequence data

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The combination of simple leaves and a dioecious sexual system found in the genus *Meryta* is unique within Araliaceae. *Meryta* comprises ca. 30 arborescent species, all endemic to one or a few islands in the Pacific, with its center of diversity in New Caledonia (11 endemic spp.). Recent family-wide phylogenetic analyses have placed *Meryta* as sister to the Pacific *Schefflera* subclade within the *Polyscias-Pseudopanax* clade, one of 3 major araliaceous lineages. A preliminary study of *Meryta* using ITS and ETS sequence data from ca. half its species provided good resolution, confirming the monophyly of the genus, in which two subclades are indicated, and indicating that 3 segregate genera are nested well within *Meryta*. A more detailed analysis with nearly exhaustive

sampling further suggests that colonization in the Pacific involved a series of long-distance dispersals to relatively young volcanic islands, primarily from New Caledonia, but also at least once from either Fiji or New Zealand.

P1310. A molecular systematic investigation of *Heracleum* L. (Umbelliferae, Apiaceae subfamily Apioideae) and related genera in the Sino-Himalayan region based on phylogenetic analysis of nuclear (ITS) DNA sequences

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The taxonomic delimitation of *Heracleum* is difficult. Recent molecular phylogenies have provisionally identified a well supported 'Heracleum clade' within the Apioideae superclade which also includes other genera. In order to test the monophyly of *Heracleum* we analysed additional ITS sequences of *Heracleum* and related genera from the *Heracleum* clade (*Pastinaca*, *Malabaila*, *Zosima*, *Tetrataenium* and *Tordyliopsis*). The data matrix is analysed using maximum parsimony. The preliminary results of our phylogenetic study confirm previous reports that *Heracleum* is not a monophyletic group, comprising at least two distinct lineages. Within the *Heracleum* clade there are at least three large clades, the core *Heracleum* clade, the *Malabaila-Pastinaca* clade and the *Zosima-Heracleum* clade. The ITS sequences provide valuable characters for inferring relationships within the *Heracleum* clade. However, to fully understand the relationships in this clade further sampling is required.

P1311. Phylogeny of Mesoamerican Vanilla: Implications for the origins of *V. tahitensis*.

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Vanilla Swartz (Orchidaceae) is a pantropical genus of about 120 species of hemi-epiphytic and saprophytic herbs. The cured fruits of only three species, *V. planifolia*, *V. tahitensis* and *V. pompona*, are commercially exploited as sources of natural vanilla. *Vanilla planifolia* and *V. pompona* are both Neotropical endemics whose centers of distribution are Mesoamerica. In contrast, wild individuals of *V. tahitensis* are unknown, and a hybrid origin between *V. planifolia* and *V. pompona* has been suggested. The evolutionary and geographic relationships among the 12 Mesoamerican *Vanilla*, and origin of *V. tahitensis* have not been examined in the context of a robust phylogeny. We sequenced both nuclear (ITS region of nrDNA) and chloroplast (non-coding regions) genomes to determine the relationships of these taxa and to test the hypothesis of a hybrid origin for *V. tahitensis*. Monophyly of Mesoamerican *Vanilla*, relationships among them, and origin of *V. tahitensis* will be discussed.

P1312. Phylogeny and Recent Differentiation in the European Alpine/Lowland Complex of *Myosotis Alpestris*

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The biogeographic history of the European alpine plant species has been only infrequently investigated using molecular methods so far. The goal of this project is to assess phylogeny of the *Myosotis alpestris* group - generally to contribute to our knowledge of the impact of Quaternary climatic changes on the plant diversity in the European mountain regions. Combining molecular phylogenies (derived from ITS region of nrDNA and from cpDNA) with information from morphology and karyology will be provided insight to the biogeographic and evolutionary history of the complex. Samples from locations throughout the European high mountains are used for analyses. The 14 populations have been examined karyologically, the diploid chromosome number for *Myosotis stenophylla* was found for the first time. Preliminary results of cpDNA analysis and nucleotide sequences of ITS regions demonstrate conspicuous difference between diploid and tetraploid cytotype. However, phylogenetic divergence within particular cytotypes is very low.

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P1312a. A molecular phylogenetic perspective on the ecology, phylogeny and life history of *Actinotus* (Apiaceae).

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Actinotus is a morphologically and geographically diverse genus of largely Australian herbs and sub-shrubs. The genus occupies a range of habitats and includes a variety of life histories and reproductive strategies. This study used chloroplast DNA sequences (*trnL-F*) to place all species of *Actinotus* within a broader, ordinal context. The *trnL-F* sequences were combined with nuclear (ETS) sequences to investigate morphological character state changes within *Actinotus*, and to raise hypotheses relating to the evolution of reproductive and life history attributes. *Actinotus* and a number of endemic Australian genera nested within a redefined "Mackinlayoideae". Several sub-clades were revealed within *Actinotus*, many correlating with geographic regions or habitats. The annual life-history, for example, evolved once within the genus and is restricted to a clade of largely semi-arid and/or fire-ephemeral species. The alpine and semi-arid species independently evolved a homogamous breeding system, along with a reduction in the number of stamens per flower. These and other insights are presented within a molecular phylogenetic framework.

P1313. Taxonomical study of diatoms of khandesh of Maharashtra (India).

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Very few workers have paid attention on taxonomical study of diatoms in Maharashtra. Diatoms are microscopic unicellular or colonial algae distinguished by the complex structure of their cell walls which are usually strongly impregnated with silica. Collections as far as possible should be made throughout the year. The diatoms are collected from puddles, ditches, lakes and river of khandesh area of Maharashtra (India). In present investigation forty two species of diatoms were recorded. In present study, the diatoms are mainly represented by the species of *Fragillaria*, *Synedra*, *Caloneis*, *Eunotia*, *Mastogloia*, *Gyrosigma*, *Anomooneis*, *Stauroneis*, *Naricula*, *Pinnularia* and *Cymbella*. In present study out of 14 genera of diatoms *Cymbella*, *Gyrosigma* and *Pinnularia* are dominant as compared to other genera. The pinnate type of diatoms were dominant over the centric diatoms in present investigation.

P1314. Epiphytic algae of some lichen species

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Data on epiphytic algae (EA) growing on lichen thalli are rather scarce and incidental. 36 samples of lichen thalli of 3 model species (*Aspicilia cinerea*, *A. contorta* var. *hoffmannii*, *Candelariella vitellina*) from granitic outcrops of Ukraine were the material of the study. Samples were provided from 12 model plots, 6 of which are situated in dry conditions of the upper portions of slope, another 6 - in wet conditions of slope along river. 43 species (Cyanoprocarota - 15, Chlorophyta - 27, Eustigmatophyta - 1) were found with the use of cultural methods. *Geminella terricola*, *Stichococcus bacillaris*, *Fottea stichococcoides*, *Desmococcus olivaceus*, *Elliptochloris bilobata*, *E. subsphaerica*, *Klebsormidium flaccidum*, found to be the most widely distributed among EA. The highest species diversity of EA is recorded for *C. vitellina* (36 species), less on *A. contorta* (26), *A. cinerea* (22). Species content of EA found to be rather close to content of lithophilic algae of granite outcrops of the same plots. Distribution of EA and lithophilic algae along the river bank slope is rather similar. But there is a number of EA, which are absent among lithophilic algal group.

P1315. Two thermophilic cyanobacteria living in the thermal baths of Montegrotto Terme (Padova, Italy)

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The therapeutical specificness of thermal mud of the euganean territory (Italy) is due to a maturation process which involves the development of a rich and differentiated biotic community of cyanobacteria and diatoms on the surface and inside the mud.

While the diatoms colonize both the surface and the entire thickness of the mud, the cyanobacteria grow mainly in the surface of the mud, forming blue-green mats.

Among the cyanobacteria, the most widespread species belong to Oscillatoriales, and among them Phormidium sp. ETS-05 is the only species up to now characterized.

In this research we report the morphological and molecular (16S and ITS rDNA) features of other two cyanobacterium strains. One of them is another form belonging to Oscillatoriales: genus Leptolyngbya. The other strain instead is a Cyanobacterium species, a new finding of the Synechococaceae (Chroococcales) in the Italian thermal muds.

These new strains were isolated from the thermal baths of the Garden Hotel of Montegrotto Terme (Padova). During the sampling the temperature in the baths was about 50°C.

P1316. The Marine Diatom Genus *Pseudauliscus* Schmidt: A Light and Electron Microscopical Study of the Diatoms *P. peruvianus* and *P. radiatus* from the northeastern Gulf of Mexico, with Comments on Lectotypification and Systematics

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Diatoms resembling *Pseudauliscus peruvianus* (Kitton ex Ralfs) Schmidt and *P. radiatus* (Bailey) Schmidt were common in collections from coastal marine habitats of Florida and Alabama over a period of several years. An examination of permanent slides from the Adams Collection (British Museum) and the Bailey Collection (Harvard) confirmed our identification of the diatoms and provided an opportunity to clarify the status of the species. Slide BM Adams GC1486 and slide 1979 from the Bailey collection were designated as the lectotypes for *Eupodiscus(?) peruvianus* Kitton ex Ralfs and *Auliscus radiatus* Bailey, respectively. SEM observations of the Florida material revealed the fine structure of ocelli and areolae, and the morphology and location of the rimoportulae in both species. The occurrence of cells of *P. peruvianus* with nuclei and plastids indicates that the species is extant and common along the northeastern coast of the Gulf of Mexico. Structural differences between the two species may require placing *P. radiatus* in a separate genus.

P1317. Phylogeny of unicellular red algae, *Cyanidium caldarium* and related algae living in acid hot springs

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Unicellular red algae, *Cyanidium caldarium* strain RK-1 and *Galdieria sulphuraria* strain M-8 were isolated from some acid hot springs in Japan. *C. caldarium* is distributed most widely in the acid hot springs, but *G. sulphuraria* is found only in the limited areas. Another unicellular red alga, *Cyanidioschyzon merolae* strain JV-95 was isolated from acid hot springs in Java, Indonesia. They have been now classified as Cyanidiaceae, Rhodophyta by some investigators. In this report, nucleotide sequences of the nuclear-encoded small-subunit 18S ribosomal RNA gene are determined on the algae, *C. caldarium* strain RK-1, *C. merolae* strain JV-95 and *G. sulphuraria* strain M-8, and their phylogenetic positions are discussed by comparing their 18S rRNA gene sequences among other algae. The phylogenetic tree of the gene indicates that *C. caldarium* and *C. merolae* form a single cluster, and *G. sulphuraria* forms another cluster within Rhodophyta. This suggests that *Galdieria* may be far from another two algae phylogenetically in more than family level. The taxonomic positions of these algae are discussed here.

P1318. The systematic review of the order *Chaetocerotales* (Bacillariophyta)

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Order *Chaetocerotales* includes diatom taxa, having special morphological element of frustule - seta (horns). In classification systems genus *Chaetoceros* was placed in family *Chaetoceraceae* as a unique one, together with *Bacteriastrum*, with *Attheya* and *Bacteriastrum*, or together with *Bacteriastrum* and *Acanthoceros*. Round et al. (1990) have put *Chaetoceros* into family *Chaetocerotaceae* Ralfs together with *Bacteriastrum* and *Gonioceros*, genera *Acanthoceros* and *Attheya* - in families *Acanthocerataceae* and *Attheyaceae*. Later, *Gonioceros* species have been transferred in genus *Attheya*. In their classification family *Chaetocerotaceae* has been considered independent and placed in order *Chaetocerotales*. In last classification system (Nikolaev, Harwood, 2000, 2001, 2002) above-stated genera and fossil genus *Chasea* were allocated into single family *Chaetocerotaceae*, into order *Chaetocerotales* together with monotypic family *Leptocylindraceae*. In my opinion, in family *Chaetocerotaceae* it is necessary to include some fossil genera.

P1319. The relationship between soil macro elements and the distribution of heterocystous cyanobacteria in paddy fields, wheat fields and a woodland in Iran.

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Despite the occurrence of paddy fields, wheat fields and woodland in Iran, less attention has been paid to the role of cyanobacteria in these ecosystems. In this study an attempt was made to growing soil inoculums on BG-11 medium. The incidence of cyanobacteria with N, P, K, C, pH and EC of soils was also determined. Totally twenty species were identified in paddy fields, twelve in wheat fields and three in woodland. The results showed that species of *Nostoc* and *Anabaena* are ubiquitous in all sites except one of paddy field stands, where its EC was the highest and *Calothrix stagnalis* was the only species found in the stand. It is suggested that this strain is probably resistance to salinity. This is supported by statistical analysis of species number and frequency with EC that showed negative correlation. The forest had higher percentage of N, P, K, and C than other regions and differed mainly in number of species and also there isn't any branching species in this region. The scarcity of algae in forest can be probably limited by low pH and EC. However soil analysis showed that phosphorous amount has a highly positive correlation with the frequency of cyanobacteria in all stands.

P1320. Depth Distribution of Epipellic Algae in Sediments of Anzali Lagoon, Iran.

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Abstract

Depth distribution and population dynamics of epipellic algal assemblages on sediments was investigated at four sites, in the Anzali Lagoon, Iran, between September 2000 and August 2001. Triplicate core samples were taken from sediments by using a sampler with 4 cm ID. The sediment was sectioned at 4, 0.5 cm intervals, and fixed by 5 ml 0.5% glutaraldehyde. Species with intact chloroplast were enumerated using Sedgwick Rafter Cell. In this study, 65 species were identified, that belong to 26 genera comprised in four classes. Species diversity of Bacillariophyceae was higher than Cyanophyceae, Chlorophyceae and Euglenophyceae. Among Bacillariophyceae *Nitzschia* with 11 and *Navicula* with 10 species had highest species diversity. The study revealed that members of Bacillariophyceae had the highest cell numbers (10^6 cells cm^{-2}) in February and members of Cyanophyceae was more abundant (140×10^3 Cells cm^{-2}) in August. Due to specific environmental conditions, 0-0.5 cm depth had more cells in comparison with other depths. Occurrence of epipellic algae in deeper sediments (1.5-2 cm) suggested that these algal assemblages might have heterotrophic nutrition.

P1321. Microphytoplankton in the marine Lake Mir, the Dugi Otok island, Croatia

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In order to characterize the microphytoplankton (MICRO) structure and abundance of the marine Lake Mir, analyses were performed in monthly intervals from November 1999 to November 2000. Sampling was carried out at one station in the lake, at the surface (0.5 m) and the bottom (7 m). The Chl a concentration was ranged

from 0-1.59 mg m⁻³. MICRO abundance ranged from 0.9 x 10³ - 6.8 x 10⁵ cells L⁻¹. Three peaks of MICRO were observed throughout the year; in November 1999 and April 2000 (mostly diatom *Cyclotella* sp.), and in July 2000 (mostly dinoflagellate *Scrippsiella trochoidea*). The MICRO abundance in the lake was similar to those recorded in the highly productive coastal zones of the Middle and South Adriatic. There was a predominance of small diatoms in spring, while dinoflagellates increased in number during summer. These patterns did not differ widely from the general succession pattern in coastal Adriatic areas and in the Mediterranean.

P1322. Epilithon structure in the Chusovaya river

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As a result of investigations of epilithon in the Chusovaya river within the summer 2003, 126 taxons of the algae of the class below the genus were identified: Bacillariophyta - 74, Chlorophyta - 37, Cyanophyta - 15. It was found that similar diatomaceous algae were prevailed in phytoperiphyton communities of stony grounds in different sections of the river.

In ecological aspect the algoflora fouling was formed by cosmopolites (69%) and boreal species (14.3%). Total composition of flora according to Kolbe's halobity system was typical for the fresh water: 66.7% were insensible to salt content in water, 14.3% were drimophilous, and 4% were halophobes. With respect to active water reaction alkaliphils (38%) and insensible (22.2%) were prevailing. Over the half of algae detected (55.7%) appeared to be the indices of water saprobity, among which the indicators of - mesosaprobic extent of contamination prevailed.

Largest biomass of algae fouling was observed on stony grounds at rifts (4.1 g/m²); it was considerably lesser on littoral grounds at reaches (1.8%), and epilithon of the reach medials was characterized by even lower quantitative development (1.1 g/m²).

P1323. Cyanoprocariota from ultrasaline littoral rock pools of the English Channel coastline

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Cyanobacteria mats were collected from supralittoral limestone rock pools at Dancing Ledge (a disused stone quarry located right on the English Channel's sea edge on the Isle of Purbeck's coast Dorset) in September 2002. The geological structure of the ledge is sedimentary limestone contains many fossils. The salinity level of these pools was from 60 to 300 psu.

A total 118 species and varieties of 38 genera from 3 orders and 8 families were identified. The assemblage is composed mainly of temperate littoral species and cosmopolitan marine species. *Caloneis*, *Lynngbya* and *Porhyrosiphon* genera present the largest number of species. The composition of the cyanoprocariota assemblage consists of two ecological groups - polyhalobous and mesohalobous species, and indicates that polyhalobous taxa dominate over the mesohalobous forms. Species as *Scytonematopsis fuliginosa* (Tild.) Kom. Et Anagn. and *Caloneis contarenii* (Zanard.) Born. Et Flah. had highest salinity tolerance (up to 300 psu) in assemblage and were highly productive. These taxa were most abundant in studied material.

P1324. *Laminariocolax aecidioides* (Phaeophyceae), epiphytic in *Undaria pinnatifida* (Phaeophyceae) in patagonian coasts of Argentina

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Laminariocolax aecidioides has been considered like an epiphytic organism of the kelps of the North Pacific, Arctic and European coasts, extending its distribution to the South hemisphere. The thalli of *U. pinnatifida* infected by this species were collected in the intertidal zone of the patagonian coasts of Argentina. On cuticle of the kelp the infection developed as dark spots of 1 - 2 mm that covered partially fronds and sporophyles. In the cortical and medullar zones of *U. pinnatifida*, the infectants cells occupied the intercellular spaces without penetrating in the host cells. The thallus of *L. aecidioides* formed a postrate layer of filaments on the surface of the host. The reproductive structures were formed in the erect system. The unilocular sporangia were uniseriate, ovoid and exhibited basal or apical dehiscence, and the pluriloculars were extended. The invasion was neither associate to

ruptures or perforations in the thalli of kelp, although it was well-known a predominance of the thalli of *L. aecidioides* related to the openings of the glands of mucilage and depressions of the epidermic layer of the host. PGI B24/077 (UNS) - PIP 2771/00 (CONICET)

P1325. Airborne fungal spores concentration and the effect of meteorological factors in Ankara, Turkey(2003-2004)

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Airborne fungal spores were collected with a Burkard spore trap in Ankara, Turkey from Jan 2003 to Jan 2004. The sum of the annual totals of the daily concentration of fungal spores, belonging to 35 taxa, were 429,264. These taxa namely Botrytis, Fusarium, Nigrospor, Pitomyces, Drechslera, Epicoccum, Torula, Stemphylium, Periconia, Curvularia, Exosporium, Spegazzinia, Tetracoccosporium, Timenticola, Dictyosporium, Melanomma, Didium, Peospora, Coprinus, Agrocybe, Gonoderma, Boletus, Puccinia, Ustilago, Didymella, Leptosphaeria, Peranospora, Xylaria, Chatemium, Venturia, Ascobolus, Melanospora, Sporomiella, Paraphaeria, Cladosporium and Alternaria. A fungal spore calendar was prepared for one year and seasonal periodicities were recorded. The results have correlated with meteorological condition. Relative humidity, temperature and rainfall showed significant fungal spores concentrations.

P1326. ITS Structure in Peronosporaceae (Oomycetes) and its possible implications for the phylogeny of Downy Mildews

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The non-coding ITS region is often screened for phylogenetic studies on and below the generic level and revealed polymorphisms and structure variation in several taxa. No such data is available for Peronosporaceae with pyriform haustoria, like *Bremia lactucae*, *Plasmopara halstedii* and *Pl. viticola*. Total ITS length was 2587bp, and a restriction-ligation technique uncovered four tandemly arranged elements (TAEs) of 322bp each and a possible marker region for these repetitive elements. The TAEs are found neither in *Phytophthora* nor in *Peronospora* spp., were ITS consists of 900bp. The TAEs showed variation in *Pl. halstedii* populations of different pathotypes, while other parts of the ITS were uniform. Other species of *Plasmopara* and *Bremia* revealed lengths of ITS up to 3kB. These lengths are derived from similar insertions, which may become a tool to trace the phylogeny of downy mildews. A comparative analysis of TAEs in *Pl. halstedii*, *Pl. viticola* and *B. lactucae* will be presented.

P1327. Geologic history of epiphyllous fungi with affinities to the Loculoascomycetes

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Symbiotic interactions in nature are important driving forces that promote diversification and influence the biogeographic patterns of the entities involved. Accordingly, parasitism and saprotrophism would not be single isolated biological interactions, but exchangeable dynamic relationships that, as influenced by the environment and the intrinsic biology of each individual involved, include all other forms of symbiosis. Although fungi have a long history, their frequent occurrence in the fossil record as dispersed individuals and the recurrent lack of preservation of their diagnostic features hinders establishment of affinities with extant members. Notwithstanding, fungi are obligate heterotrophic organisms that are dependent on the presence of other biological individuals for existence. As a result, the occurrence of fungi in past ecosystems may have been determined by the presence of certain taxa of other organisms. In this contribution, the geographic distribution of epiphyllous fungi throughout the Tertiary and part of the Mesozoic is evaluated and correlated with the occurrence of host plant taxa and particular climatic conditions.

P1328. Endophytic fungi in *Brosimum alicastrum* (Moraceae) and *Hampea trilobata* (Malvaceae) leaves in Calakmul Biosphere Reserve, Campeche, México.

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It is expected to found in tropical regions most of the undiscovered endophytic fungal species (Spyreas *et al.*, 2001) because the high diversity of plant species and vegetation types (Fisher *et al.*, 1995) represent a broad potential substrate and habitat.

The study was carried out at the Calakmul Biosphere Reserve, the biggest and best preserved in Mexico (Martínez *et al.*, 2001), sampling leaves of *Brosimum alicastrum*, a dominant and well distributed species in Mexican tropical forests (Pennington and Sarukhán, 1998) and *Hampea trilobata*, an endemic species in the Yucatan peninsula (Fryxell, 1988).

Tree leaves were surface sterilized, and endophytic fungi were isolated with specific culture media. Strains identification was made with traditional taxonomic methods.

We found more than 30 endophytic species, 306 strains were isolated from *Brosimum alicastrum* with 27% colonization frequency and 388 from *Hampea trilobata* with 33% colonization frequency. It is also presented a host preference analysis and a comparison of the found fungal species with those reported in other plant species from tropical regions (Rodrigues 1994, Arnold & Herre 2003).

P1329. Cryptic basidiomycetes in a crustose lichen symbiosis

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Crustose lichens of the *Lecanora rupicola* group are the host of several lichenicolous fungi and lichenicolous lichens. Apart from these other accessory fungi were detected in thallus areoles during a phylogenetic investigation of the *Lecanora rupicola* group. Apparently, these fungi may live more or less cryptically and without external symptoms of degradation as endolichenic fungi in lichens. So far no sexual structures were detected and the classification of these fungi relied on molecular sequence analyses. Diverse fungi of the Tremellales are involved in such associations, but none of them belong to groups already known to occur on other lichens. A posteriori inspection indicates that infected thallus areoles are slightly more convex than the adjacent areoles to hypertrophized. Symptomless fungal infections of lichens by basidiomycetes may possibly be a widespread, but underestimated phenomenon.

P1330. Biodeterioration of archaeological textile dyes by the fungi and bacteria in Iran

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Introduction: we have assessed the potential of fungi and bacteria and a laccase from this organism to continuously degrade textile dyes. Material and methods: 100 samples were isolated from archaeological textile of Iranian museum and in 2004 in Iran. We have kept them in 5-20 days, one sample was chosen for control. Result: we have succeeded to recognize fungi and bacteria species: *Ba subtilis*, *Ps aeruginosa*, *Thio thiooxydans*, *Ph chrysosporium*, *Pe chrysogenum*, *Geo candidum*, *As foetidus*, *As niger*, *As flavus*, *Trich rezezi*, *Ceph acremonium*, *Rhiz delemar*, *Pe restericum*. Finally we have researched activities of laccase thereon textile in *Ba subtilis*, *As foetidus*, *As niger*, *Ph chrysosporium*, substrate (2-6 dimethoxy phenol). Beginning of discoloration in the first several days and decreasing of colorization after one week in *As niger* and *As foetidus*, after two weeks in *Ph chrysosporium* and after 20 days in *Ba subtilis*. Conclusion: Activities of laccase were studied with spectrophotometric examination in different times and recoded according to wave length. We have observed in *As niger* 428 nm that 90% is discolored, in *Ph chrysosporium* 370 nm that 71% is discolored.

P1331. Geosmithia fungi - dry spored entomochoric fungi associated with bark beetles, description of diversity, host specificity and pathogenicity

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Phloemophagous bark beetles are vectors of different fungi. We have studied bark beetles associated with the fungi of the genus *Geosmithia*. We collected 162 wood samples infested by 40 bark beetle species from 150 locations in central Europe and Mediterranean. Isolates of *Geosmithia* spp. (about 1000) were classified into 23 morphotypes characterized by micromorphology and RAPD patterns (RAPDtypes). Some RAPDtypes were

determined as *G. putterillii* and *G. lavendula* and several new species was described. It was confirmed that most studied bark beetles (*Ernoporus tiliae*, *Hylesinus* spp. *Hypoborus ficus*, *Scolytus intricatus*, *S. rugulosus*) carry specific range of RAPDtypes. Vector specificity of geosmithias proves that these fungi are entomochoric. In *G. lavendula*, we discovered its highly specialized vector, *Hypoborus ficus*, parasitizing fig trees in Mediterranean. Geosmithias are primary colonists of a fresh phloem rich in sugars and are not able to utilize complex carbon sources. Some tested cultures strongly inhibited *in vitro* root and stem growth in young plants of garden cress; therefore phytopathogenic potential of geosmithias cannot be excluded.

P1332. Fungal diversity in *Calluna vulgaris* (L.) HULL

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Biologically active secondary metabolites are produced by numerous endophytic fungi and may be involved in fungus-host plant interactions as well.

The diversity of *Calluna vulgaris* (Ericaceae) inhabiting fungi was characterised by molecular methods. Fungal strains were grouped by ITS/5.8S nrDNA sequence data and the 18S nrDNA was sequenced for representatives of each ITS genotype. The sequences were aligned with all publicly available high quality SSU nrDNA sequence data of Ascomycetes.

A range of individual host plants derived from different habitats and physiologically standardized clonal plants were used.

Molecular phylogenetic analyses of endophytic as well as epiphytic fungi revealed a wide spectrum of fungal phylotaxa at the host-specific and site-specific level.

The production of secondary metabolites by endophytic fungi in plants was characterized by various phytochemical methods. In addition, endophytic fungi obtained from mycelial outgrowth from surface-sterilized healthy-looking host tissues, were tested for their antimicrobial and enzymatic properties in culture.

P1333. The effects of the disaccharides on *Morchella conica* Pers. mycelium development

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The effects of the disaccharides on *Morchella conica* Pers. mycelium development

Morchella conica mycelium was used, sucrose and maltose was chosen as disaccharides and 0.25%, 0.50%, 0.75%, 1.00% and 1.25% concentrations have been added to wheat agar, potato dextrose agar, malt extract agar and complete medium yeast agar. The radial growth speed, morphologic specifications, radial growth ratios, colonization period and pigmentation of mycelium were taken as criteria.

The development period of mycelium in wheat agar was completed in 4 days and the mycelium were very thin. The colonization period of the mycelium were determined; 7 days in potato dextrose agar, 5 days in malt extract agar and 5 days at complete medium yeast agar. The development of the mycelium; at potato dextrose agar was dense and circular, at malt extract agar and in complete medium yeast agar was rhizomorphic. Mycelium had developed very well at sucrose medium and formed creamy and light yellow pigmentation. At maltose medium the mycelium development was good and light yellow and light brown pigmentations were noticed.

Key Words: *Morchella conica*, mycelium development, disaccharide.

P1334. Pneumocystis nomenclature

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Pneumocystis, causal agent of a pneumonia (PCP) in immunocompromised individuals, has defied classification. Initially, it was mistakenly included in the lifecycle of the protozoan *Trypanosoma cruzi* causing Chaga's Disease. Separately described, *Pneumocystis* was believed to be a protozoan covered by the zoological code. Having only 3 codes, botanical, zoological and bacteriological, bizarre nomenclatural situations arise when the 3

kingdom system applies to microorganisms, e.g. rumen chytrids, mycetozoans, microsporidians. Molecular phylogenies reveal new relationships and taxa can 'switch' kingdoms, resulting in cascading nomenclatural changes. *Pneumocystis* exemplifies one extreme because it combines 'misclassification' in the 'animal' kingdom and initial inclusion in the lifecycle of a protozoan that led to the mixed element description of *Schizotrypanum* and its type, *T. cruzi*, representing two 'kingdoms'. Additionally, reduced morphology and host-specificity hinder subgeneric classification causing debate regarding the name for the human pathogen, *P. jirovecii*, often called *P. carinii*. Nomenclatural resolutions are detailed.

P1335. *Buellia subalbula* - studies of a complex species group from semi-arid to arid regions

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Preliminary studies indicate that *Buellia subalbula*, as previously often identified, represents a species group of anatomically, morphologically and chemically, similar, but not identical taxa from semi-arid to arid regions. All taxa have one-septate ascospores and heavily pruinose, rimose thalli containing large amounts of Ca oxalates. The type specimen from Angola contains norstictic acid and an aeruginose apothecial pigment reacting strongly violet with HNO₃. Exciple and epihymenium of specimens from the Mediterranean, coastal Baja California and Southern California are less strongly aeruginose, but still show the distinct reaction. In contrast, the reaction is almost undetectable or entirely absent from a group of Australian specimens that are not strictly confined to coastal habitats. Another group of Australian specimens lacks both norstictic acid and the aeruginose pigment, but instead contains xanthonenes. Some of these thalli seem to be parasitic on a thin, gray, inapparent thallus containing only norstictic acid and showing no trace of the aeruginose apothecial pigment. The taxonomic status of all these taxa will be further investigated.

P1336. Biological diversity of lichens in Caucasus and their protection

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The study of the Caucasian lichens began more than 200 years ago. During these years, more than 300 works on lichenology and geobotany have been published. At present time on the territory of Caucasus 1300 taxons are known which are related to 126 kinds and 49 families.

The Caucasus in its rich nature has extremely various landscape, geological structure, climate, orography which promoted the development of specific flora here, including the discovery of the new lichens. For the last century 65 new taxons have been described from Caucasus. The new species as a genofund of aboriginal flora must be included in the "Red Book of Azerbaijan". Among the lichens of Caucasus there are species, which are under the threat of local disappearance and even general destruction.

The rapid change of the surrounding cenotic situation under the influence of the abiotic, biotic and especially, anthropogenic factors on the Caucasus, brought to the disappearance of many relicts, endemic species. Some of them are left as a herbarium, others reduced their areal.

We hope, that the XVII International Botany Congress will play an important role in solving of this problem.

P1337. Katalalixar: Liverworts Paradise

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The rainy-temperate forests of Chile hold a very rich core of biodiversity, with a special richness of cryptogams, often exceeding that of vascular plants. The taxonomic singularity and biogeographical interest of the bryophytes associated to these forests, particularly in Katalalixar, have a direct relation to the long geological history of the continent and oceanic climate. The liverwort diversity in the studied area concentrates for more than the 50% in the forest floor, setting up a surprising biomass which also entirely covers tree trunks up to 5-6 meters and 20 cm thick. In addition, an important number of species grow on rocks and logs in the intertidal zone of the fjord system. Phytogeographically, a 57% of the species is endemic to the forested ecosystems of

southern South America, while the subantarctic element represents a 27%. At generic level, a 42% of the flora has a wide distribution on the globe, while only the 8% results to be endemic. The distribution of the species restricted south to 48° lat S and the species having disjunctions to the northern Chile and the Neotropics have a particular interest.

P1338. '*Bartramia stricta*' Brid. (Bartramiaceae) in the Mosel valley - a relict from the postglacial temperature optimum?

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The Mediterranean moss *Bartramia stricta* Brid. was first discovered in Germany in 1932 and subsequently recorded from a dozen localities in the Mosel valley area. Molecular studies were started with the aim to test its status as relict from the postglacial temperature optimum (10,000 yrs b.p.). Phylogenetic analyses based on sequence data of chloroplast trnSGGA - trnFGAA region revealed that the sterile populations have nothing in common with the Mediterranean, British or North American populations of *B. stricta*. In contrast, a close relationship to populations of *Anacolia laevisphaera* (Tayl.) Flow. in Grout is indicated by the molecular analyses. Moreover, the German populations are morphologically identical to *A. laevisphaera*. Therefore, the German specimens have to be referred to *A. laevisphaera*, a tropical montane species. This is a first example of a bryophyte species which has been added new to the flora of Europe due to a molecular study.

P1339. Molecular variation in *Isothecium alopecuroides* (Lam. ex Dubois) Isov., including a deviating form from Morocco

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In course of identification of epiphytic bryophyte samples from Morocco, an *Isothecium* with characteristics of *I. alopecuroides* (Dubois) Isov. and, to a smaller degree, *I. algarvicum* W.E. Nicholson & Dixon was found. Problems with attributing the plant to any *Isothecium* species, and the known large morphological variation in *I. alopecuroides*, suggested that molecular studies were needed to evaluate patterns of relationships in this complex.

A total of 87 samples were studied, of which 12 belong to the Moroccan plant, 56 to *Isothecium alopecuroides* from different regions of Europe, 1 to *I. alopecuroides* from China, 2 to *I. algarvicum*, and the rest to other *Isothecium* species, which were used as outgroup: 2 of *I. subdiversiforme* Broth., 4 of *I. myosuroides* Brid., 4 of *I. myosuroides* var. *brachythecioides* (Dixon) C.E.O. Jensen, 2 of *I. stoloniferum* Brid., 1 of *I. cristatum* (Hampe) H. Rob. and 3 of *I. holtii* Kindb.

The rRNA-Gly and ITS markers were investigated and revealed molecularly distinct populations of *I. alopecuroides* in Morocco, Eastern Europe, and some Western and Northern European localities.

P1340. A revision of the genus *Thamnobryum* (Musci) of Japan

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A taxonomic revision was made on Japanese species of the genus *Thamnobryum*, based on morphological and molecular data. Eight species were recognized to occur: *T. alopecurum* (Hedw.) Nieuwl. ex Gangulee (*Thamnium planifrons* Broth. & Yasuda, syn. nov.), *T. coreanum* (Cardot) Nog. & Z.Iwats., *T. subseriatum* (Mitt. ex Sande Lac.) B.C.Tan, *T. cymbifolium* (Cardot) Kagenishi, stat. nov. (*Thamnium sandei* var. *cymbifolium* Cardot; *Thamnium sandei* var. *angustifolium* Nog., syn. nov.), *T. coreense* (Cardot) Kagenishi, comb. et stat. nov. (*Thamnium sandei* var. *coreense* Cardot; *Thamnium sandei* var. *imbricatum* Nog., syn. nov.), *T. plicatulum* (Sande Lac.) Nog. & Z.Iwats., *T. incurvum* (Nog.) Nog. & Z.Iwats., and *T. neckeroides* (Hook.) E.Lawton (New to Japan!). The following characters were newly recognized to be of important taxonomic criteria additional to the characters previously evaluated for the distinction of species: papillosity of leaf cells, cell wall thickenings of stem, structure of costa, and sexuality. A key to the Japanese species was given.

P1341. The complex thalloids - evolving simply

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A molecular phylogeny was produced for 36 species (26 genera) from the Marchantiopsida and two species from the Blasiaceae, and rooted using seven species from *Haplomitrium*, *Treubia* and *Apotreubia*. Sequence data were generated for the chloroplast genes *rbcL*, *rps4* and *psbA*, the nuclear ribosomal LSU and the mitochondrial gene and intron *nad5*.

Combined analyses of these support the monophyly of the complex thalloids and the sister relationship of the Blasiaceae. There is little support for early branches within the Marchantiopsida, although the Sphaerocarpaceae resolve as the first diverging lineage.

Lunularia and *Neohodgsonia*, taxa with complex archegoniophores, also diverge early, while simpler taxa lacking archegoniophores, i.e. *Riccia*, *Ricciocarpos* and *Monoclea*, nest deep within the group, implying morphological reduction in some of the more phylogenetically advanced lineages. A *Marchantia/Preissia* (Marchantiaceae) clade is supported as sister to all remaining complex thalloids. The families Cleveaceae and Aytoniaceae are monophyletic, but their largest genera *Athalamia* and *Asterella* are not.

P1342. Reconstructing Evolution in the Metzgeriales Schljakov subord. Pallaviciniineae R. M. Schust., the "Vascularized" Liverworts

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Multigene analyses of the simple thaloid liverworts resolve a monophyletic clade comprising the Hymenophytaceae R. M. Schust. and Pallaviciniaceae Mig., a synapomorphy of which is the differentiation of strands of elongate, hydrolyzed cells in their gametophytes. Six well supported lineages are resolved within this clade, but the genera *Pallavicinia* Gray, *Symphyogyne* Nees & Mont., *Moerckia* Gottsche and *Podomitrium* Mitt. are paraphyletic. To test whether there are morphological characters that are better predictors of the evolutionary relationships within the group than those currently used to define genera, a combined analysis of molecular and morphological data has been conducted, and morphological character evolution has been reconstructed on the combined tree. Oil body morphology, spore wall architecture and some features of perichaetial organization are predictive of several of the paraphyletic lineages, while variations in strand cell anatomy are not. Sporophyte-investing structures and capsule wall anatomy are phylogenetically informative, but there is substantial homoplasy in perigonal organization and plant habit.

P1343. A Revision of the Colombian species of *Polytrichadelphus*

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A revision of the Colombian species of *Polytrichadelphus* (Polytrichaceae: Musci) is presented. The genus is very variable morphologically. Seven species are recognized in Colombia: *Polytrichadelphus abriaquiae*, *P. aristatus*, *P. ciliatus*, *P. giganteus*, *P. longisetus*, *P. purpureus* and *P. valenciae*; they are distributed mainly in cloud forest and paramo vegetation, and are adapted to a number of different substrates.

A numerical taxonomic study of the genus was carried out. Cluster Analyses with the unweighted pair group method using arithmetic averages (UPGMA), as well as ordination methods (Principal Component Analysis) were used to establish phenetic relationships among species and to obtain the most important characters for their delimitation. A total of 20 morphological characters were studied in 114 Colombian samples of the genus, of which 92 were used for the numerical analysis.

An artificial identification key to the Colombian species is provided. The following information is given for each taxon: morphological description, geographical distribution (with maps), ecology, and general observations. Each species is illustrated and a list of specimens examined is included.

P1344. A revision of the genus *Dimerodontium* (Bryophyta) with considerations about familiar position using cpDNA

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The genus *Dimerodontium* was firstly described by William Mitten in his work entitled "*Musci Austro Americani*" in 1869. Between the year 1877 and 1956, a total of 14 names were added to this genus. Even though the genus has some characters distinct from Fabroniaceae, its original familiar position was never challenged. For the first time a comprehensive study including a monograph of the genus and molecular phylogeny based on cpDNA is presented. A new definition of the genus is proposed with three species *D. mendozense* Mitt., *D. pellucidum* Mitt. and *D. balansa* Müll Hal. Eleven new synonyms are also presented. New *rbcL* sequences were obtained for the genus *Dimerodontium* and *Fabronia*, the remaining sequences used in the study were downloaded from the GenBank. Data were analyzed under maximum parsimony and maximum likelihood criteria using PAUP 4.0.b10. Results under both criteria do not support a monophyletic Fabroniaceae as traditionally circumscribed; *Dimerodontium* appears as sister group of a clade containing *Fabronia*, *Gollania*, *Abietinella*, *Stereophyllum* and *Entodontopsis*.

P1345. Molecular cytological comparative analysis of *Pellia* species (Hepaticae).

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Pellia borealis is one of the first described allopolyploid liverwort, originated after hybridization of two allopatric taxa *P. epiphylla* S and *P. epiphylla* N. Allopolyploid origin of *P. borealis* is well documented by molecular and isoenzyme markers, but cytogenetic evidences are still lacking. Detailed chromosome analyses of polyploid and ancestor genomes have been carried out. Haploid gametophyte of *P. epiphylla* S and *P. epiphylla* N has 9, while *P. borealis* 18 chromosomes. Differential chromosome staining like DAPI/CMA3 and C-band allowed identifying some chromosomes of progenitors in hybrid karyotype. That confirmed allopolyploid origin of *P. borealis*. Only one locus 26S rDNA was determined on *P. borealis* chromosomes by FISH. It is supposed that some rearrangements occurred in polyploid genome and locus of rRNA genes from one progenitor have been lost. Flow cytometry estimation of genome size of studied species showed that *P. borealis* nuclear DNA content is almost sum of *P. epiphylla* N and *P. epiphylla* S content.

P1346. Anatomical and ultrastructural innovations in *Leiosporoceros dussii* (Steph.) Hässel

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The genetic isolation of *Leiosporoceros dussii* from all other hornworts is corroborated by unique morphological features, which are detailed in this study. *Nostoc* occurs in median schizogenous canals that run the length of the thallus and are distinctly different from the globose colonies of other genera. The cyanobacteria invade sporelings through stomata-like clefts near the growing tip; no clefts are visible in older plants. *Nostoc* strands elongate and branch in synchrony with apical growth. Sporophytes contain extensive sporogenous tissue with small, smooth spores in isobilateral tetrads. The abundant pseudoelaters are highly elongated with thick cell walls. Spore wall deposition is initiated by plates of tripartite lamellae, giving rise to the external exine that is subsequently obscured by sporopollenin. This first report of tripartite lamellae in hornworts solidifies this feature as a synapomorphy for land plants. When molecular and morphological data are considered collectively, *Leiosporoceros* provides essential clues in reconstructing the evolutionary history of bryophytes.

P1347. Brotherus' and Fleischers' view of the Lembophyllaceae: better than molecules?

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During the last century the moss family Lembophyllaceae has undergone fundamental changes in its familial delimitation, and yet

the generic and familial level relationships are still poorly understood. Originally, the Lembophyllaceae contained four genera: *Lembophyllum*, *Camptochaete*, *Dolichomitra*, and *Isothecium* (Brotherus 1909). Later, Brotherus' concept of the Lembophyllaceae broadened, and by 1925 he included another 8 genera. This resulted in a seemingly heterogeneous assemblage being criticized by several later researchers arguing that the included genera show very little similarity. Here we present a generic revision of the Lembophyllaceae based on *trnL-F*, *psbT-H* and ITS2 sequence data that clearly reveals that the current circumscription of the family is too narrow and that several genera, which have been placed in other families, such as *Bestia*, *Dolichomitra*, *Dolichomitropsis*, *Isothecium*, *Fallaciella*, *Rigodium*, and *Tripterocladium*, should be re-included in the Lembophyllaceae.

P1348. A molecular phylogeny of the genus *Leucobryum* (Leucobryaceae, Musci) in Asia and the Pacific

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Leucobryum includes ca. 180 species, and is widely distributed in the world. Yamaguchi (1993) monographed Asian *Leucobryum*, and noted several important taxonomic characters for species recognition. However, there still remains the taxonomic ambiguity of the circumscription of species. The aim of the present study is to determine the phylogenetic relationships among species of *Leucobryum* in Asia and the Pacific. Our study corroborates evidence that 1) *Leucobryum* is a monophyletic entity including some species of *Schistomitrium*; 2) four major clades: the *L. juniperoideum*-clade, *L. scabrum*-clade, *L. bowringii*-clade, and *L. sanctum*-clade can be recognized; 3) the *L. sanctum*-clade was morphologically distinguishable from the other three clades by leaves with distinct auriculate alar regions; and 4) the distribution of perichaetia on the stem, the proration of abaxial leaf cells at leaf apex, and the central strand of stem were proved to be important characters which reflecting phylogeny.

P1349. Botanical Diversity

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Mosses which play a key role in the formation of natural biotic community & indicative of forest condition were abundant at Khandala and Mahabaleshwar in the *Western Ghats of India*. Eversince Sedgwick (1909), Dixon (1911) & Bruhl (1931), etc. - research on mosses was neglected. In addition to this scenario, forest of said localities have been succumbed by human interference. Survival of many mosses have been threatened. Therefore an attempt has been made to reinvestigate them. It appears that Mahabaleshwar possess 30 terricolous, 27 saxicolous, 34 corticolous, 5 lignicolous, 1 aquatic saxicolous mosses. Khandala possess 16 terricolous, 16 saxicolous, 15 corticolous, 2 lignicolous taxa. Some of them indicate high degree of adaptability in their habitat. Fruiting Archidium indicum, Diaphanodon procumbens were new to this region. Moss Flora of Mahabaleshwar is much more rich than that of Khandala due to conducive climatic condition such as high altitude (4500 Ft.) & high rainfall (200 inches).

P1350. Origin and subdivision of *Plagiochila* (Jungermanniopsida: Plagiochilaceae) in tropical Africa

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Plagiochila with about 450 species is the largest genus of Hepatics. Centre of diversity are the humid tropics of Asia and tropical America. The *Plagiochila* flora of tropical Africa is rather poor, possibly as a result of extensive drought periods over the Pleistocene which could have resulted in the extinction of parts of the local hepatic flora. Phylogenetic analyses of chloroplast gene *rps4* and nrITS sequences of *Plagiochila* lead to a well supported topology with African species nested in six predominantly tropical clades. The majority of African species belongs to *Plagiochila* sect. *Vagae*. Several species occur both in the Neotropics and Africa. ITS sequence similarities indicate that their ranges are the result of long distance dispersal rather than vicariance. Molecular data provide some evidence for long-range dispersal events eastwards across the Atlantic, originating from the Neotropics.

P1351. Taxonomic and phylogenetic positions of the genera *Brotherella*, *Pylaisiadelpha* and *Wijkia* (Sematophyllaceae)

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Although often discussed, the taxonomic position of the genera *Brotherella* Loeske ex M.Fleisch., *Pylaisiadelpha* Cardot and *Wijkia* H.A.Crum (Sematophyllaceae) is still unclear. The monophyly of these three genera is tested, and *Wijkia* appears as a polyphyletic entity. Analyses of morphological and phylogenetic data show that only *Wijkia extenuata*, the type of the genus *Wijkia*, which occurs in continental Australia, Tasmania, and New Zealand, should be treated as a member of the subfamily Heterophylloideae of the family Sematophyllaceae. It is suggested that Asian species of *Wijkia* should be treated as members of the genus *Brotherella*. Alar cells proved to be an important character in separating *W. extenuata* from other species of the genus *Wijkia*. We consider that the Sematophyllaceae should be subdivided into three subfamilies: Sematophylloideae, Heterophylloideae, and Pylaisiadelphoideae.

P1352. Molecular Systematic Study of *Conocephalum* (Hepaticae)

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Bryophytes are small land plants with a simple morphology. Therefore, we can predict the existence of several biologically distinct units or cryptic species within the morphological species of bryophytes. In our previous study, we found three rbcL types in Asian *Conocephalum japonicum* (Thunb.) Grolle and also got several evidences strongly suggesting that these types are cryptic species which are reproductively isolated (Miwa et al. 2003, 2004). In this study, we tried to examine rbcL sequence variations from 6 cryptic species of *C. conicum* (L.) Dumort. formerly recognized by allozyme analyses (Odrzykoski and Szwejkowski 1991, Akiyama and Hiraoka 1994). As a result, we could discriminate the six cryptic species based only on their rbcL sequences. In addition, we found a sample of *C. conicum* which is probably a new cryptic species from Sakhalin because it has quite distinct rbcL sequence from any of the formerly known 6 cryptic species. It was shown that rbcL sequence variations were also useful in finding cryptic species of *C. conicum*.

P1353. Bryophytes on cliffs and talus slopes of south-western Moravia (Czech Republic)

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Bryophyte flora of rock habitats and talus slopes was studied in south-western Moravia (Czech Republic). The total number of species was 140 (23 liverworts, 117 mosses) for cliffs and 93 (20 liverworts, 73 mosses) for talus slopes (26 study sites). The most frequent species on both habitats were *Dicranum scoparium*, *Hedwigia ciliata*, *Hypnum cupressiforme*, and *Pohlia nutans*, on cliffs also *Cephaloziella divaricata*, *Ceratodon purpureus*, and on talus slopes also *Barbilophozia barbata*, *Grimmia ovalis*, *Pleurozium schreberi*, and *Polytrichum formosum*. Ten threatened bryophytes were recorded for cliffs and four for talus slopes. Temperate and subboreal chorological types were the most frequent for cliffs and temperate, boreal, and subboreal on talus slopes. Among the growth-forms, rough mat and short turf types were the most often both on cliffs and talus slopes. The most frequent life strategies were perennials and colonists both on cliffs and talus slopes.

P1354. Phylogeny of *Grimmia* Hedw. (Grimmiaceae, Bryopsida) : reproductive strategies influence recombination rates

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In this work we investigate the phylogenetic relationships among 33 species of *Grimmia* Hedw. using two plastid DNA sequences (trnL-trnF & rps4) and 52 morphological characters. The combined analysis of the sequence and morphological data and using Funariidae and Dicranidae species as outgroup supports the monophyly of the family Grimmiaceae and indicates that *Grimmia*, as currently defined in literature, is paraphyletic. Two clades are present in the tree, one containing the species traditionally placed in the subgenus *Rhabdogrimmia* Limpr. and one containing the remaining *Grimmia* species. These clades obviously differ neither in their geographical nor ecological characteristics. An analysis, using 120 trnL-trnF sequences and representing the 33 *Grimmia* species, shows a difference in the intraspecific variability of the two clades: 'Rhabdogrimmia' contains asexual well-defined species with little intraspecific variability compared to *Grimmia* s.l. which is composed by sexual less-defined species with higher intraspecific variability. These two different reproduction strategies may imply two different recombination rates.

P1355. A new species of *Exormotheca* (Exormothecaceae, Hepaticae) from China

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A new species of *Exormotheca* (Exormothecaceae, Hepaticae) is reported from China. This is the first record of Exormothecaceae from China and the easternmost locality of the family in Eurasia. The field research organized by the National Science Museum, Tokyo, was made in the southwestern Sichuan Province, China, in cooperation with the Kunming Institute of Botany in 1996. During the field survey in the area of Mt. Singuniangshan unique marchantioid plants were found. The thallus has strongly elevated air chambers coupled with simple air pores and cubical carpocephala of archegoniophores, which are characteristic of the family Exormothecaceae. The family consists of three genera, *Aitchisoniella*, *Stephensiella* and *Exormotheca*, of which *Aitchisoniella* and *Stephensiella* are monotypic and endemic to the western Himalayas. Sichuan plants are a member of *Exormotheca* because of having chlorophyllous filaments in air chambers, and are distinct from other species of the genus by short septa of air chambers, rounded ventral scales with entire margin, ciliate appendages and presence of oil-cells. Sichuan plants may also belong to Sect. *Corbierella*.

P1356. Moss flora in the greater area of Belgrade

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The research of the moss flora in the greater area of Belgrade included large greens in fifteen localities: the Danube quay, Rušanj, the Sava quay, Avala, the forest of Bojčinska, Kanarevo brdo, Surcin, Banjica, Ada Ciganlija, Zvezdara, Košutnjak, Višnjica, Topcider, Ilicev dunavac, Monastery forest in Rakovica.

The research has proven the existence of 182 moss species which have been classified in 75 genera and 27 families. According to literature (Sabovljevic, 2000) the number of 182 taxons of mosses represents 34% of the moss flora in Serbia.

The research into the moss flora of the greater area of Belgrade has brought to light 8 species that were not previously found in the moss flora in Serbia. They are: *Brachythecium latifolium* Kindb., *Brachythecium campestre* (C. Mull.) B.S.&G., *Brachythecium starkei* (Brid.) B.S.&G., *Ditrichum pusillum* (Hedw.) Hampe, *Fissidens exilis* Hedw., *Rhynchostegiella tennella* (Dicks.) Limpr., *Isopterygyopsis muellerana* (Schimps.) Iwats. and *Tortella fragilis* (Drum.) Limpr.

The research has pointed to a great diversity of moss species and habitats in which they develop.

P1357. Genetic Diversity Of Some Selected Economically Important Philippine *Alliums*

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The present study covered interspecific genetic diversity of *Alliums* commonly grown in the Philippines such as *Allium ascalonicum*, *Allium cepa*, *Allium chinense*, *Allium fistulosum*, *Allium odorum* and *Allium sativum*. Eight morphological characters were used such as bulb mass and diameter; number of scales and cloves; diameter, height and mass of cloves; and leaf appearance (either flat or cylindrical) to construct a dendrogram. The RAPD analysis yielded 64 loci all of which are polymorphic. The largest genetic distance coefficient is between *A. fistulosum* and *A. sativum* which is 0.693147. The first clade consisted of *A. fistulosum* and *A. chinense*, which had a bootstrap value of 52%. The second clade was composed of *A. ascalonicum* and *A. cepa*, with a bootstrap value of 99%. *A. sativum* was paraphyletic relative to *A. fistulosum* and *A. chinense* with a bootstrap value of 73%. *A. odorum* was divergent relative to the rest of the *Alliums*, with a bootstrap value of 100%. The clade of *A. cepa* and *A. ascalonicum* deviated from the clade of *A. fistulosum*, *A. chinense* and *A. sativum*. This study is useful for the selection breeding and species conservation of Philippine *Alliums*.

P1358. Intraspecific variability of the species of the genus *Allium* L. 1754 (Amaryllidales, Alliaceae) of the southern part of the Pannonian Plains (Serbia and Montenegro)

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Since the determination with standard keys is based upon the morphological characters, the obtained results point to an insufficient number of characters used in intraspecific differentiation, in particular when taxa ranked as varieties and forms were discussed. Determination with standard keys was impracticable in certain cases, despite the fact that certain situations pointed out their belonging to the individual taxa. The analysis of all the morphological characters used in determination of intraspecific *Allium* taxa of the southern edges of the Pannonian Plains was done to perform adequate and more practical determination. Data were statistically processed using Statistica software for Windows version 5.1. ANOVA and MANOVA tests using taxon as a factor, Principal Componente Analysis (PCA), and Discriminant and Correspondence Analysis were also done. Combinations of characters for intraspecific taxa determination were separated. Cluster analysis showed a similarity between variation of quantitative and qualitative characters.

P1359. Phylogenetic analyses of the genus *Crinum* (Amaryllidaceae) with emphasis on tropical African species.

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Fifty species of *Ammocharis*, *Crinum* and *Cybistetes* were sequenced, with *Amaryllis* chosen as outgroup. Two main clades were revealed: All *Ammocharis* taxa, *Crinum baumii* and *Cybistetes longifolia* constituted the first main clade and the rest of the *Crinum* taxa constituted the second. The second main clade is split into three subclades. The first subclade comprises almost only taxa with starshaped flower ("Stenaster"). The second subclade includes taxa, all with bellshaped flowers ("Codonocrinum") with main distribution in Southern Africa. The third subclade includes "Codonocrinum" taxa with tropical distribution. Within this subclade a monophyletic group including the rest of the "Stenaster" taxa is situated (those dist. in W. Africa and America). Interestingly a newly discovered species *Crinum binghamii*, found in Zambia, links in a basal position in this W-African to American group. There is accordingly no support for taxonomic recognition of subgeneric delimitation related to flower morphology, but, there are strong geographical and ecological trends in the phylogeny, which will be discussed further.

P1360. Molecular Systematics of The Genus *Goniothalamus* Hook. f. & Thomson

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The genus *Goniothalamus* Hook. f. & Thomson is one of the largest and most important genera in the Annonaceae. Its members are widespread mainly in South Asia and South East Asia. Many *Goniothalamus* species hold potential as important medicinal resources in Thai traditional medicinal practices, including, for example, the roots of *G. macrophyllus* (Blume) Hook. f. & Thomson. Little is known of the evolution and biogeographical history of this genus. Many morphological characters are ambiguous and reveal little phylogenetically useful information. Molecular systematics, nonetheless, provides tools that can answer these questions. Preliminary results show that the sizes of *rp16* intron, *trnL* intron and ITS in all species studied are about the same (i.e., 1,200, 580 and 750 bp, respectively). ITS, however, has the most phylogenetically informative sites, although the size of ITS is shorter than *rp16* intron. Of all three loci, ITS locus yields the highest number of phylogenetically informative sites, whilst cp markers give few sites. Moreover, DNA fragments from ITS have greater nucleotide substitution than the others.

P1361. Reassessing the generic status of *Petalolophus* (Annonaceae): evidence for the evolution of a distinct sapromyophilous lineage within *Pseuduvaria*

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The genus *Petalolophus* (Annonaceae) consists of only one species, *P. megalopus*, and is characterized by large perianth wings that extend abaxially from the midrib of the inner petals. Recently published molecular phylogenetic data suggests that *Petalolophus* is congeneric with *Pseuduvaria*. Morphological and anatomical characteristics of both genera are critically re-examined. *Petalolophus* only differs from *Pseuduvaria* by the autapomorphic possession of perianth wings, indicating that *Petalolophus megalopus* should be transferred to *Pseuduvaria* as "*Pseuduvaria megalopus*". Unlike most Annonaceae, *Pseuduvaria* species are fly-pollinated with inner petals that are apically connivent, forming an open chamber around the reproductive organs. Many *Pseuduvaria* species have light-coloured petals (usually cream or pale yellow), whereas other species are presumably sapromyophilous since they have dark red, purple or brown patches on the petals, and often emit an unpleasant odour. The dark red, convoluted perianth wings of *P. megalopus* are unique in the genus as they clearly mimic carrion, and field observations show that the flowers are visited by flies.

P1362. The genera *Anthericum* and *Chlorophytum* (Anthericaceae), evolution and generic delimitation

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Altogether 70 accessions representing 40 species of African and European *Chlorophytum* and *Anthericum* were analysed by sequencing ITS and *trnL-F* spacer and running cladistic analyses with *Leucocrinum* as outgroup. The taxa are grouped in several clades, but the basal topology of the three shows little resolution. However, some clades, obviously with taxonomic sense, were revealed: European *Anthericum* constituted one, and African *Anthericum* another monophyletic group. African *Chlorophytum* taxa that up to mid 1990s were referred to *Anthericum* constituted more than one clade. The taxa formerly referred to *Dasystachys* belongs in one clade. All taxa defined by basal chromosome number $x=7$, including most rain forest taxa, constituted a large, poorly resolved clade. This cladogram indicates that, the genus *Chlorophytum* has transgressed from savanna into rain forest at least twice. Generic delimitation and, also delimitation of some species will be discussed, but more data are needed to conclude.

P1363. The Middle Asia as the greatest diversity center of ephemeroïd Umbelliferae (Apiaceae) in the Old World

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Ephemeroïd Umbelliferae are the perennial herbaceous monocarpic or polycarpic plants with tuberous or vertical thickened storage roots. They usually become dormant towards the middle of summer. This plant group is presented in arid areas and is common in the Mediterranean region.

By the recent data, 449 species of Umbelliferae belonging to 109 genera have been recorded in the Middle Asia, including Kazakhstan, Kyrgyzstan, Uzbekistan, Turkmenistan and Tadjikistan. About half of them (196 species from 24 genera) are ephemeroïds. *Ferula*, the largest genus of Umbelliferae in the Middle Asia (96 species), contains ephemeroïd species mainly. These plants have vertical or napiform storage roots. The plants belonging to such large genera of Umbelliferae, as *Elaeosticta* (19 species) and *Bunium* (17 species) have usually storage roots in the form of globose geophilic tubers.

The largest number of ephemeroïd Umbelliferae in the Middle Asia belongs to Apieae tribe. At the same time, such large genera as *Bupleurum* and *Seseli* do not contain ephemeroïds at all.

P1364. Major clades within Apiaceae subfamilies Saniculoideae and basal Apioideae: Implications for evolution of the woody habit.

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The family Apiaceae, as traditionally circumscribed, comprises three subfamilies, with the largest, Apioideae, being the focus of much systematic attention. In this study, we expand results of a previous phylogenetic analysis of chloroplast *rps16* intron sequences by incorporating additional representation of "core Apiaceae" (Apioideae, Saniculoideae, and the *Azorella* clade) with several woody or herbaceous African umbels, many of which have been postulated to be links between subfamilies Apioideae and Saniculoideae, or to be important lineages in the early evolution of core Apiaceae. Among basal Apioideae, two additional major lineages are inferred (*Annesorhiza* clade; *Lichtensteinia*); tribe Heteromorphae is expanded. Saniculoideae is maintained as sister to *Steganotaenia* plus *Polemanniopsis*. Core Apiaceae is also expanded to include a fourth major clade (*Hermas*, treated previously as a hydrocotyloid genus). A southern African origin of subfamilies Apioideae and Saniculoideae is postulated, and the placement of herbaceous members at the base of Apioideae suggests, surprisingly, that its ancestor was herbaceous, not woody.

P1365. Architecture and fruit set effects in *Chaerophyllum bulbosum* (Apiaceae)

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Most of the European Apiaceae-Apioideae are andromonoecious and show rather constant sex ratios. To test the plasticity in sex expression biennial *Chaerophyllum bulbosum* was used as a model system and a field trial was carried out in a natural population on the Rhine river banks near Mainz, Germany. The plants bear about 80% (functionally) male flowers in a total of 1000 to 35000 flowers that are produced in white umbels in up to four umbel orders. Treatments were i) control, ii) additional hand-pollinations, iii) exclusion of insects by bagging entire inflorescences and iv) removal of first and second order umbels. Bagged individuals produced both perfect and male flowers. Individuals with removed umbels had significantly more perfect flowers in the umbels of higher branch order and even produced an additional branch order. The results show that andromonoecy in *Chaerophyllum bulbosum* is an architectural effect which sets the constraints of sexual lability in individual flowers depending on their position and total fruit set.

P1366. Leaf shape variation in *Monstera adansonii* Schott and *M. praetermissa* E.G.Gonç. & Temponi (Araceae) from eastern South America

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Leaf shape variation was studied in *Monstera adansonii* and *M. praetermissa*. The study is based on over 1500 leaves from 28 field populations in Brazil (Pará, Ceará, Pernambuco, Bahia, Espírito Santo, Rio de Janeiro, São Paulo) and French Guiana. Shapes were compared by multivariate analysis (PCA, CVA) of the Elliptic Fourier coefficients obtained from digitized leaf outlines. Shape/size correlation was studied using principal component scores as shape attributes and correlation between perforation

number and leaf blade length was investigated. *M. adansonii* is distinguishable from *M. praetermissa* on leaf blade shape alone, using mature leaves, pre-mature leaves or both together. Many populations were distinguishable within *M. adansonii*, often when geographically close, but the pooled populations did not partition into consistent subgroups. Model-based cluster-analysis resulted in three groups of *M. adansonii* populations, with *M. praetermissa* remaining separate. Shape of pre-mature leaves is just as useful as the mature shape; this result could be useful for conservation field surveys in humid forest islands in Northeast Brazil.

P1367. A Phytogeographic Analysis of Araceae of Cabo Corrientes (Chocó Department, Colombia) and Comparable Lowland Tropical American Floras

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We studied the Araceae of the Cabo Corrientes region on the Pacific Coast of Colombia, and compared its aroid flora with those of La Selva (Costa Rica), Barro Colorado Island (Panama), Bajo Calima (Colombia), Río Palenque (Ecuador), and Iquitos (Peru). We found 113 species in 14 genera at Cabo Corrientes; the largest genera were *Anthurium* (38 species), and *Philodendron* (35), which together accounted for 64 percent of the species. Seventy five percent of the species were exclusively epiphytic or hemiepiphytic. Most of the epiphytic species belonged to the genera *Anthurium*, *Philodendron*, *Rhodospata*, *Syngonium*, *Monstera*, and *Stenospermatum*, the latter three containing exclusively epiphytic or hemiepiphytic species. Five of the wild species are used locally. The flora of Araceae of Cabo Corrientes was most similar to that of La Selva, Barro Colorado Island, and Bajo Calima; similarity with Río Palenque was low. Our findings support Lellinger's view that the Chocó biogeographic region extends to the Nicaragua-Costa Rica border and it is divided into a northern and a southern floras.

P1368. Molecular and morphological systematics of the *Tetraplasandra* group (Araliaceae): floral diversity and implications for pollinator shifts

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Tetraplasandra, *Reynoldsia*, and *Munroidendron*, are closely related genera distributed in Samoa, French Polynesia and the Hawaiian archipelago. Maximum parsimony analyses based on ITS, 5S-NTS, AFLPs, and morphology indicate a monophyletic Hawaiian lineage (the *Tetraplasandra* group), a sister-taxon relationship between *Munroidendron* and *R. sandwicensis*, and a polyphyletic *Reynoldsia*. Variation in floral morphology corresponds to phylogenetic relationships within and among the genera. Within *Tetraplasandra*, species with hypogynous flowers (*T. gymnocarpa*, *T. kawaiensis*) cluster together and species (*T. waimeae*, *T. waialealae*) with floral characters suggesting bird pollination cluster together. The differences in floral morphology may be associated with a switch to ornithophily. Field studies confirm native and non-native birds feeding on the blossoms of *T. waimeae* and *T. waialealae*.

P1369. *Helwingia* and other genera at the turn of *Cornales* and *Apiales* (Araliales) - evidence that fruit anatomy can provide

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The position of some genera in *Cornales* has been questionable. One of them is *Helwingia*, often related to *Araliales* (as monotypic *Helwingiaceae*, or *Helwingiineae* within *Araliales*, or even order *Helwingiales*, close to *Araliales*). Our carpological study revealed new facts to support this treatment, namely: *Helwingia* fruit type and general structural pattern markedly resemble those in most *Araliales* (the number of pyrens in *Helwingia* is reduced to 2-4). Some differences though exist (no secretory reservoirs in *Helwingia* pericarp, different seedcoat structure). Our research demonstrated that out of *Cornales* representatives, usually related to *Araliales*, *Mastixia* has features of resemblance with *Araliaceae*, showing it as an archaic taxon: mesocarpium secretory ducts, associated with vascular bundles; endocarp structure.

Never related to *Araliales*, *Kaliphora* has dimeric half-inferior pyrenarium with literally discrete endocarp (pyrens are not completely fused); slightly ruminated endosperm of *Kaliphora* was never found in any *Cornales*, whereas reported for *Araliales*.

P1370. Molecular phylogenetics of the palm genus *Chamaedorea*

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We conducted the first molecular phylogenetic analysis of *Chamaedorea* (Arecaceae: Ceroxyloideae: Hyophorbeae), the largest neotropical genus of palms. The 100+ species of *Chamaedorea* have not been analyzed cladistically, nor has the monophyly of the genus previously been tested. The prevalent current taxonomy of *Chamaedorea* recognizes eight subgenera (Hodel 1992), all based on floral characters. Sequence data from the low-copy nuclear genes PRK and RPB2 were analyzed, and *Chamaedorea* was found to be monophyletic with strong support. Three of the smaller subgenera are well supported by the molecular data. However, at least two of the subgenera are non-monophyletic including the two largest, *Chamaedorea* and *Chamaedoropsis*, which are distinguished from each other by the degree of connation in the staminate petals. Many of the well-supported clades resolved by these gene regions are unsupported by existing morphological evidence and define groups never before proposed within *Chamaedorea*. Two large clades are identified that would benefit from further study, as they contain morphologically diverse and interesting species.

P1371. A Phylogeny of the Palm Tribe Hyophorbeae Based on Plastid DNA sequences

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In this work we conducted the first phylogenetical analysis of the tribe Hyophorbeae (Arecaceae), using molecular data from 4.5 kb of coding and noncoding plastid DNA (*matK*, *rps16* intron, *ndhF* and *trnD-trnT*). The tribe Hyophorbeae is a monophyletic group including more than 115 species. The tribe has a disjunct distribution with four genera in Central and South America and one genus on the Mascarene Islands. The placement of this tribe within the palm family has been controversial, but the tribe is now firmly placed in subfamily Arecoideae. However, the relationships of Hyophorbeae with other groups of the subfamily are still unclear.

In our analyses all five genera in Hyophorbeae are resolved as monophyletic with high support, however, the relationships among the genera are not clear. The monotypic genus *Wendlandiella* from South America and the genus *Hyophorbe* from the Mascarene Islands form a monophyletic group sister to the remaining three genera. Despite the general difficulty to get resolution at low taxonomical level in palms using plastid DNA, we were able to resolve some of the species level relationships, especially within the largest genus *Chamaedorea*.

P1372. The Micromorphology of leaf in *Aristolochia* (Aristolochiaceae) genus from Guerrero, México.

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On basis of reproductive structures the genus *Aristolochia* has been partitioned in two subsections *Hexandrae* and *Pentandrae*. The aim of this project is to describe the micromorphological characteristics founds in the leafs of 21 species of *Aristolochia* genus inhabiting in Guerrero state, México. The methodological approach involves leaf fragments of 1 x 1 cm from under and upper surfases, these samples were treated with a thin cover of gold in a Denton Vacuum Desk II Cold Sputter. The samples were observed with a SEM Joel JSM-5310LV. It was found that the main types of trichomes are: Straights, uncinated and the combination of these uncinated and straight in the same leaf; there were monocellulars and pluricellulars trichomes with a composition of two to nine cells.

Almost all the species under study have uncinated and straight trichomas except *Aristolochia veraguensis* of *A. inflata* that only have uncinated trichomes with two or three cells. Finally it was found that the pubescence isn't useful as characteristic for identify species in the genus *Aristolochia* at leaf not in all the cases, but in some of them.

P1373. Microsporogenesis of *Thitonia diversifolia* (Hemsl.) Gray (Asteraceae-Heliantheae)

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Thitonia is an American genus of the Asteraceae family, currently constituted by 40 species and infraspecific taxa; in Venezuela, only *Thitonia diversifolia* (Hemsl.) Gray and *T. rotundifolia* (Mill.) S. F. Blake, have been reported. *T. diversifolia* is used in medicine, as green manure, and in weed control. Little is known about its cytology. The study was made with inflorescences collected from different localities in Monagas state, Venezuela, with anthers of early flower buds, fixed in absolute alcohol-acetic acid (3:1) and coloured with acetic orcein (2%). In this species, the microsporogenesis was observed to be simultaneous. Abnormal meiosis divisions were also observed, with lagged chromosomes in metafase I and anaphase I, and asynchronic chromosomes in metafase II. The pollen grains were 26.5µm long, triporate type and muricate sculpture, 64% of them without spermatid nuclei. These irregularities may be responsible for the low percentage of pollen fertility found.

P1374. Local adaptations of rare species *Aster amellus* L. (Asteraceae)

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Many recent studies showed negative effects of lower population size on plant fitness. One possibility to increase population size is to transfer individuals from other populations. While this approach has many advantages, it can also have negative effects in case of strong local adaptation. Several previous studies showed local adaptations in species over large geographical scales. Nothing is, however, known about local adaptations of nearby populations, over which the transfers are most likely to be done.

We study local adaptations among populations of self-incompatible perennial species of dry grasslands. We selected three populations in two different regions in central part of the Czech Republic and carried out transplant experiments both within and between regions. This enabled us to estimate the degree of local adaptations at two spatial scales. We also analysed allozyme variation of these populations and abiotic conditions and vegetation composition of their localities. Thus, we could test whether genetic and environmental differences of the populations correlate with the degree of local adaptation.

P1375. *Argyranthemum Webb ex Schultz Bip.* (Asteraceae: Anthemideae). Morphological and Reproductive Biodiversity Micromarkers in an Endemic Macaronesian Genus

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The reproductive systems of plant species are considered to be a determining cause of biodiversity at natural population level, where the reproductive characteristics control the reproductive success of crosses and biodiversity configuration, both genetic and morphological.

Floral organization has been the principal basis of taxonomy and systematics to the extent that systematics and reproductive biology have been closely allied throughout botanical history because of the implications for both of reproductive characters through time and space. Their integration principally at infrageneric levels is indispensable and has high potential value.

This study centres on 9 taxa from Gran Canaria of genus *Argyranthemum* which is made up through adaptive radiation of 24 species and 15 subspecies. The objective is to estimate and understand biodiversity within an island through the detection of morphological reproductive micromarkers and genetic diversity, the inter-intrapopulation structure and its correlation with interpopulational geographical distances. At the same time, the autoincompatibility and therefore the xenogamy of the genus is confirmed

P1376. Anatomic characteristics of vegetative organs of the Balkan endemic species *Aster albanicus* Deg.

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Abstract. Morphological and anatomic characteristics besides genetic basis represent the real reflection of their adaptability against ecological factors of environment where they are realizing its own life. In this direction we approached to the research of vegetative anatomic organs building on endemic species of Kosovo and Albania *Aster albanicus* Deg., extended in Koznik (Kosova) and Divjakë (Albania).

The preparation of materials for research of anatomic building of root, stalk and leaves has been done under the standard methodology of paraffining and the usage of shipping microtome.

The researches show that there do exist the clear differentiation in anatomic building of leaves (palisade tissue, sponger, number of stomas and nervous density) individuals from Koznik and Divjaka. On basis of analyzed measuring can be concluded that the *Aster albanicus* Deg., type belongs to plant group with mesomorph characteristics but presenting the kseromorph characteristics too, especially at individual of *Aster albanicus* in Koznik taking into consideration the conditions of locality kserotoms where are spread out.

P1377. Biogeographic Patterns and Evolution in the Asteraceae of the Páramos

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Páramos or high-elevation floristic communities (>3000 m) occur in Costa Rica, Panama, Venezuela, Colombia, Ecuador, and extreme northern Peru and contain 101 families and ~3214 species of flowering plants. Asteraceae is the largest family with 113 genera & ~ 973 species (30%) including Senecioneae (277 spp), Astereae (208 spp), Heliantheae (192 spp), Eupatorieae (98 spp), Gnaphalieae (77 spp), Mutisieae (32 spp), Lactuceae (30 spp), Liabeae (25 spp), & Barnadesieae (11 spp). Senecioneae is most diverse (23%), followed by Astereae (13%), and Heliantheae (13%). *Westoniella* and *Jessea*, are restricted to Costa Rica and Panama, and 15 genera do not reach northern Peru, e.g., *Espeletia*, *Ascidogyne*, *Chersodoma*, *Helogyne*, *Novenia*, *Mniodes*, & *Paranephelius* lack species N of 4°-6° S latitude. *Baccharis*, *Diplostegium*, *Ferreyrella*, *Jalcophila*, *Loricaria*, *Luciliocline*, *Lucilia*, *Senecio*, *Werneria*, & *Xenophyllum* are throughout the Andes. Phylogenetic studies in *Paranephelius* (*Liabeae*) (7 species, of which 5 are in N Peru) utilizing ITS sequence data suggest that *Paranephelius* is monophyletic, and with *Pseudonosseris* constitute the *Paranepheleinae*.

P1378. Evolution by Reticulation of the *Acrolophus* subgroup (*Centaurea* L., Compositae) in the occidental Mediterranean. Origin and diversification of the section *Willkommia* Blanca

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Section *Willkommia* Blanca (genus *Centaurea* L., Compositae) is a group of morphologically very similar taxa which is endemic of the middle east of the Iberian Peninsula and northwest of Africa. According to Heywood (1960) and Blanca (1981) its diversification was by a schizoendemic process from an ancestral singameon. Sistematically it is included in the *Jacea* group, *Acrolophus* subgroup, together the sections *Acrolophus* (Cass.) DC. and *Phalolepis* (Cass.) DC.

Comparative analysis of the ITS and 3'ETS regions of the nrDNA reveals the presence of three different ribotypes in the *Acrolophus* subgroup: *Willkommia*, *Acrolophus-Phalolepis* and *Simulans*. These ribotypes show a clear geographic structuration which doesn't correspond with the Systematic of this subgroup. We explain this conflict among the structuration of the genetic diversity and the morphological evidences by a reticulate evolution model of the occidental Mediterranean taxa.

We also postulate the hybrid origin of the *Willkommia* ribotype and the occurrence of the ancestor of the *Willkommia* section associated to the hybridization process which originated the ribotype.

P1379. ITS phylogeny of the genus *Centaurea*: the *Jacea* group

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The new delineation of the genus *Centaurea*, now circumscribed for the major part to the large group of species with *Jacea* pollen type, is the reason for a new molecular study of this group with the aim of verifying the sectional classification and the relationships between sections. We have analyzed the ITS sequences, which we had used in a preliminary study, on an extended sample of the group, and we have compared the resulting molecular phylogeny with present sectional classification. Our results confirm that there is a narrow correlation between molecular data and geographic distribution. We have unraveled unsuspected close relationships between some sections, and we have cleared up the position of some species previously not classified in any section. Finally, a large polytomy and the anomalous placement of some taxa in a complicate group of sections from the Eastern Mediterranean region strongly suggest that reticulation has played an important role in the evolution of this group.

P1380. Genus *Centaurea* L. (Asteraceae) in Georgia

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The *Centaurea* genus, which comprises approximately five hundred species, is mainly distributed in the countries of Mediterranean, also in Central and East Europe and Near East. About a hundred and ten species were found in the Caucasus.

The specific composition of the genus was specified for Georgia, the system of the genus was elaborated on the level of subspecies based on geomorphologic, polynogical, and anatomic carpological methods.

The representatives of Georgian flora are grouped into eleven subspecies. Reasoning from the concept of polytypical species, twenty-five species are named for the flora of Georgia.

The nature of some species' endemism, as well as small local populations of limestone ecotopes (in the Western Caucasus), could be assumed as a basis for establishing the conservation status category of the species, meeting the requirements of the International Union for the Conservation of Nature (IUCN) and providing reserves for the conservation of the given species and protected territories.

P1381. The Tribe Anthemideae Cass. (Asteraceae) in the Caucasus

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A critical revision of the tribe Anthemideae during more than 25 years has specified its taxonomical composition in the Caucasus. The study was carried out by morphological-geographical method and the use of carpological-anatomical data.

On the basis of studying huge herbarium material (LE, ERE, TBI, BAK, MHA, G, E, P, K, B, FL etc.), type specimens, authentic and photocopies of types, personal herbarium collections and observations in nature during numerous field surveys it was revealed that the Anthemideae in the Caucasus is represented by 9 genera and 97 species. The Anthemideae composition in the Caucasus is as follows: *Anthemis* L. - 16 species, *Achillea* L. - 14, *Anacyclus* L. - 1, *Artemisia* L. - 22, *Leucanthemum* Mill. - 1, *Matricaria* L. - 3, *Othanthus* Hoffm. et Link - 1, *Tanacetum* L. - 29, *Tripleurospermum* Sch.Bip. - 10.

Nomenclature, typification, geographical distribution, endemism, altitude and ecological conditions as well as practical significance of species were clarified. 11 rare species were revealed out of which 9 species are narrow endemics and 2 - considered extinct.

P1382. Biosystematic studies in the genus *Senecio* (Asteraceae)

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The genus *Senecio*, a member of Asteraceae includes over 1500 species. The global distribution of *Senecio* indicates its ancient origin. The present investigation involves 12 species of *Senecio*

and discusses their interrelationship from the point of view of flavonoid spot pattern on the chromatogram. On morphological grounds, taxa studied fall into four groups. This grouping of species is assessed in terms of numerical systematics based on flavonoid data. The parameters are paired affinity index, matching coefficient, negative correlation and biochemical distance. Findings from these support the grouping of the species on morphological grounds. Two other statistical parameters - Group affinity (GA) value, and Isolation value (IV) are used to study the intrageneric relationship of the species. Low GA and high IV indicate one of the possibilities- a) The genus is highly plastic and under selection pressure there is a maximum diversity within a short period. b) The genus may be ancient and during evolution, the genome has undergone numerous changes leading to diversity in characters. It is concluded that *Senecio* is ancient in origin and its species are diversified.

P1383. Comparison of ribosomal DNA site patterns on somatic chromosomes in ten *Aster* species and two allied genera (Asteraceae)

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Somatic chromosomes of ten *Aster* species and two putative-allied genera of *Erigeron* (*E. thunbergii*) and *Solidago* (*S. virgaurea*) in Japan were investigated by the aceto-orcein staining method and the fluorescent *in situ* hybridization (FISH) method using 45S ribosomal DNA (rDNA) probes. The ten *Aster* species, *E. thunbergii* and *S. virgaurea* commonly showed the chromosome number of $2n = 18$. In karyotype, *A. asa-grayi*, *A. miyagii* and *A. spathulifolius* classified to the section *Pseudocalimeris* showed similar karyotype to each other, while *A. komonoensis*, *A. rugulosus*, *A. savatieri* and *A. scaber* classified to the section *Teretiachenium* did not show consistent karyotype. In FISH, the ten *Aster* species and *S. virgaurea* exhibited an rDNA site at the secondary constriction of two chromosomes. *Erigeron thunbergii* exhibited an rDNA site at the secondary constriction and whole satellite of two chromosomes. The rDNA distribution patterns of the *Aster* ten species were more similar to that of *Solidago* than that of *Erigeron*.

P1384. A study of achene slime in several taxa of *Artemisia* (Asteraceae, Anthemideae)

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Artemisia is one of the largest genera in the Asteraceae, with ca. 500 taxa, from perennial shrubs to annual herbs. It is basically distributed in the Holarctic region and often dominant in deserts, semi-deserts and steppes. It propagates mainly by achenes, characterized by the presence of slime, clearly visible after hydration. We examined the distribution of the slime cells on the achene surface, the slime structure and its formation in connection to plant life form and habitat. The *Artemisia* slime belongs to the cellulose type and consists of pectins and cellulose. Our results reveal the pattern of slime cell distribution, which differs between taxa. A large slime amount, rapidly formed on the achene surface, is typical of plants living in dry habitats and of annual species. The slime facilitates germination and fruit adhesion to the ground or to animals. Thus, it may play an important role in the distribution and expansion of *Artemisia* taxa, being an adaptive feature to dry habitats.

P1385. Genus *Artemisia* L. (Asteraceae) in Georgia

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A genus *Artemisia* L. spread in Georgia is one of the critical and complex genera of the family Asteraceae, which is due to its systematic structure and geographic distribution.

In Georgia the genus *Artemisia* L. includes species, which participate in various vegetation formations and are connected with various belts. They are distributed up to 3000 m above sea level (from the lower mountain belt to the subalpine and alpine ones). Species of the genus occur on dry stony slopes, rocky and

skeleton ecotopes, roadsides, croplands as weeds. Some species are edifiers of the semi-desert vegetation.

12 species of the genus *Artemisia* L. spread in Georgia are arranged in 3 subgenera on the basis of the shape and structure of the vegetative and generative organs.

Subgenus I: *Artemisia* - 1. *A. vulgaris*, 2. *A. chamaemelifolia*, 3. *A. armeniaca*, 4. *A. annua*, 5. *A. caucasica*, 6. *A. splendens*, 7. *A. austriaca*, 8. *A. absinthium*, 9. *A. incana*

Subgenus II: *Dracunculoides* - 10. *A. marchalliana* = *A. campestris*, 11. *A. scoparia*

Subgenus III: *Seriphidium* - 12. *A. lerchiana* = *A. fragrans*

P1386. A study of the taxa *Scorzonera* L. and *Podospermum* D.C. (Asteraceae) in Italy using cytogenetical and molecular tools (sequencing of ribosomal ITS and ETS regions).

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Morphological differences between the two taxa *Scorzonera* L. (of which there are thought to be 12 species in Italy) and *Podospermum* D.C. (3 species in Italy) are quite small - leaves in the latter are pinnate rather than lanceolate or spatulate, and the base of the achene is concave rather than straight. As a consequence, there has been some debate about their taxonomic status; should *Podospermum* be considered a distinct species or as a subgenus of *Scorzonera*? In addition, some of the relationships among species of *Scorzonera* are not entirely clear. The aim of this study was to analyse all 15 species found in Italy using both cytogenetic and molecular tools (ribosomal ITS and ETS sequences) in order to help clarify these relationships. Results are consistent with an interpretation that places both taxa into one genus. All species, except *S. hirsuta* and *S. villosa* having only 12 chromosomes, were $2n = 14$. *S. hirsuta* and *S. villosa* were also the most distinct molecularly, and formed a well supported distinct group. The 3 species of *Podospermum* clustered with the other species of *Scorzonera*, being most closely related to *S. aristata* and *S. humilis*.

P1387. Tetraploid and octoploid cytotypes in *Senecio jacobaea* (Asteraceae) in the Pannonian Basin and Carpathians

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Populations of *Senecio jacobaea* L. subsp. *jacobaea* in the Pannonian Basin and in the Carpathians were studied using karyological and morphological approach. Two different ploidy levels were revealed by flow cytometric analyses in the area studied: tetraploids and octoploids. Tetraploids (distributed commonly throughout Europe) have been found as widespread both in Pannonia and in the Carpathians. Octoploids have been found only in Pannonia and in adjacent mountains of the West Carpathians. In the sympatrical zone of both cytotypes (Pannonia and adjacent parts of the West Carpathians) mixed populations with both tetraploid and octoploid individuals have been identified. Results of the morphometric analyses showed that the ranges of the morphological characters (number of tubular florets, length of tubular florets, length of involucre bracts, number of involucre bracts, length of ray florets, indumentum of outer achenes) of both cytotypes broadly overlap. It was not possible to distinguish the cytotypes reliably by any quantitative character or by their combination.

P1388. Genetic diversity and differentiation in the diploid populations of *Dendranthema zawadskii* and its related species (Asteraceae)

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Although *Dendranthema zawadskii* and its related species have relatively clear taxonomic criteria, their phylogenetic relationships have not been understood well because of the lack of diploid individuals for molecular investigation. However, we could collect diploid individuals of two species (*D. zawadskii* and *D. indicum*) from Korea, and conduct molecular phylogenetic and allozyme

analyses to see relationships among the diploid populations of *D. zawadskii*, *D. indicum* and *D. boreale* that are distributed in East Asia. The cpDNA trees showed that populations of the same species did not form a single clade in any species; in other words, any clade was composed of populations of different species. We found even that populations of different species had the same haplotype. Also, the dendrogram obtained by allozyme data generated three major clusters, each of which was composed of populations of different species. Therefore, morphological species concept was not supported. Molecular evidence rather suggests that hybridization have frequently occurred among the populations of the different species of *Dendranthema*.

P1389. The system of the genus *Senecio* L. and *Tephroseris* (Reichenb.) Reichenb. of the flora of Georgia

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Species of *Senecio* L., a genus characteristic to the flora of Georgia, are presented by considerable components of mountain and high-mountain florocenotypes. The genus *Senecio* L. has been studied in complex. On the basis of macro-morphological and micro-morphological (pollen morphology and seed coat anatomy) characteristics, circumscriptions of the genus are established for Georgia's flora. Genus *Senecio* sensu stricto is limited. *Tephroseris* (Reichenb.) Reichenb. is considered as independent genus. Genera *Pojarkovia*, *Iranecio*, *Caucasalia* are considered as synonyms for the genus *Senecio*. In the genus circumscription new sections and subsections [Gen. *Senecio* - Sect. *Ologanthi*: Subsect. *Albidiflori* (Gagnidze) Gagnidze et Gagaa, Subsect. *Platiphylli* (Gagnidze) Gagnidze et Gagaa; Sect. *Mediterranei* Gagnidze et Gagaa, Sect. *Scitophylli* (Gagnidze) Gagnidze et Gagaa; Gen. *Tephroseris* - Sect. *Cladobotrytes* (Gagnidze) Gagnidze et Gagaa; Sect. *Primulifolii* Gagnidze et Gagaa] are described, a new nomenclature combination is provided.

P1390. The evolution of B-class genes in diploid and allotetraploid *Tragopogon*

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Tragopogon mirus Ownbey and *T. miscellus* Ownbey are recent allotetraploids ($2n = 4x = 24$) that formed repeatedly during the past 80 years following the introduction of three diploids (*T. dubius* Scop., *T. pratensis* L., and *T. porrifolius* L.) from Europe into eastern Washington and Idaho, USA. Despite the frequency of polyploidy in plants, little is known about the expression and fate of genes duplicated by polyploidization. We examined homologs of the B-class floral regulators *APETALA3* (*AP3*) and *PISTILLATA* (*Pt*) in these tetraploids and their diploid parents. Comparative expression studies examined the contribution of the *Pt* homologues of the parental species to the two allopolyploids. *T. miscellus* has two different copies of *Pt* homologues. The nucleotide sequences from *T. dubius*, *T. mirus*, and *T. porrifolius* were identical, except for differences in the 3'-untranslated region.

P1391. Taxonomic study of *Filago* L. (Asteraceae: *Gnaphalieae*) in Iran

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Filago is classified in the tribe *Gnaphalieae* (Asteraceae). The tribe *Gnaphalieae* is one of the largest in the family, with more than 180 genera and 2000 species. *Filago* contains 35 species in north temperate areas, 12 in Iranica area, and 9 species in Iran. *Filago* is divided to three subgenera: Subgen. *Filago*, Subgen. *Oglifa*, and Subgen. *Evax*. All the subgenera have some species in Iran. Only subgenus *Evax* has sterile hermaphrodite flowers in the middle part of the capitules; the number of the bracts in subgenus *Filago* is more than the subgenus *Oglifa*.

In this study all the Iranian species taxonomically were deeply studied. For them morphological characteristics were determined. Identification key is prepared for the Iranian species based on many studied specimens from TARI, FAR, IRAN, TUH, and B herbaria.

Between many collected Iranian plants, one specimen is new to science. The specimen is collected from West Iran (Azarbaijan and Kordestan Provinces). It is closely related to *F. arvensis*.

P1392. Towards an understanding of the trigger plant flora (*Stylidium*) of South West Australia

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With over 250 taxa, the trigger plant genus *Stylidium* (Stylidiaceae) is amongst the most abundant and diversified genera in Australia; however, this group has received significantly less attention than the iconic Australian genera *Eucalyptus*, *Acacia* and *Grevillea*. Whilst several accounts of *Stylidium* were published in the 19th and early 20th centuries by botanists in Europe, no formal revision of the genus has been undertaken by a botanist based in Australia. Substantial progress has recently been made toward such a treatment for the *Flora of Australia*. This research has focused on taxa from South West Australia: a region internationally recognised for its biodiversity, and a major centre of *Stylidium* diversification. Twenty-five new species have been identified, many of which are geographically restricted and of conservation concern. Field studies have been pivotal to this research since many taxonomically informative characters are difficult, if not impossible, to discern on herbarium specimens. The location and interpretation of historical type material and the correct application of early botanical names remains a major research challenge.

P1393. Morphological variation of *Lactuca serriola* populations from some European countries

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Because of a great importance of *L. serriola* in commercial lettuce breeding, the best attention has been paid to effective germplasm management of this species in last decades. Fifty *L. serriola* populations collected in four European countries were cultivated in a greenhouse under controlled conditions and morphologically characterised following the descriptor list of wild *Lactuca* spp. germplasm. As for leaf morphology only *L. serriola* f. *serriola* was recorded in the Czech Republic (100% of plants), 91% of plants originated from Germany were determined as form *serriola* as well. Populations collected in the Netherlands consisted of plants determined as form *integrifolia* in 54.4% of individuals while in populations collected in the United Kingdom it was 98% of plants. Results concerning the other morphological and developmental characteristics obtained during the study are discussed. Funded by projects: EU GENE-MINE (Contract No.QLK5-2000-00722) and MSM 6198959215.

P1394. *Betula*, *Corylus* and *Alnus* airborne pollen in Zagreb, CroatiaR. Peternel¹, B. Miti ², J. ulig¹, I. Hrga¹, P. Hercog¹;¹Zagreb Institute of Public Health, Zagreb, Croatia, ²Faculty of Natural Sciences, Zagreb, Croatia.

The aim of this study was to build up a picture of the influence of meteorological conditions on the start and duration of Betulaceae pollen season and pollen concentrations during three years (2002-2004) in the atmosphere of Zagreb, Croatia. A 7-day VPPS 2000 Hirst volumetric spore trap was used for pollen sampling. The highest annual pollen concentration of all three genera (*Alnus*, *Corylus* and *Betula*) was recorded in 2002 (3,883 pg/m³, 11,106 pg/m³ and 14,448 pg/m³), followed by a decline in 2003, and rise again in 2004. The highly favorable weather conditions in 2002 during the period preceding the pollination of alder, hazel and birch (January) resulted in earlier flowering time of these plants and thus earlier occurrence of their pollen in the air. In all three years, pollination peaks were recorded on the days characterized by temperature above 0 °C and no or minimal precipitation.

The results of the present study provide useful data for allergologists to reach accurate diagnoses, and timely information on airborne pollen types and concentrations for individuals with pollen hypersensitivity.

P1395. Factors influencing sexual expression in the fern *Woodwardia radicans*

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The gametophytes of homosporous ferns are potentially bisexual. However, depending on growing conditions, gametophytes can develop into male, female or dichogamous. In some species, like *Woodwardia radicans*, asexual gametophytes become male in the

presence of female and bisexual ones because of the effect of pheromones (i.e. antheridiogens).

Sexual expression is size-dependent in flowering plants and also likely in homosporous ferns, although studies are scarce. In *W. radicans*, male gametophytes are smaller than female and bisexual gametophytes, but the factors affecting size and sexual expression are poorly known. We experimentally manipulated density of individuals, and light and nutrient availability in order to study the resulting sexual expression. Our aim was to find out (1) whether poor growing conditions, independently of antheridiogen, induce maleness, (2) whether a threshold size exists for the change from male to female sexual expression and (3) whether that threshold size is flexible or fixed.

P1396. On the taxonomy of Brachytheciaceae (Musci) in China

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The present paper studies the taxonomy of Chinese moss family Brachytheciaceae. The studies are based on field studies, type specimens and collections in Chinese and other herbaria. The research results shows there are eighty species of the Brachytheciaceae, belonging to the eleven genera in China. These genera and species number for each genus are as follows: *Camptothecium* (2 species); *Tomenthypnum* (1 species); *Homalothecium* (2 species); *Palamocladium* (2 species); *Brachythecium* (43 species); *Bryhnia* (5 species); *Cirriphyllum* (2 species); *Myuroclada* (1 species); *Eurhynchium* (11 species); *Rhynchostegium* (9 species) and *Rhynchostegiella* (2 species). Keys to genera in the family and species for each genus based on some important gametophyte and sporophyte characters are also provided together with illustrations and geographical distribution for all Chinese species.

P1397. Morphological- Reproductive Biodiversity Micromarkers in the Canarian Endemic Genus *Parolinia* Webb (Brassicaceae).

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The reproductive systems of plant species are considered to be a determining cause of biodiversity at natural population level, where the reproductive characteristics control the reproductive success of crosses, gene flow and biodiversity configuration, both genetic and morphological.

Floral organization has been the principal basis of taxonomy and systematics to the extent that systematics and reproductive biology have been closely allied throughout botanical history because of the implications for both of reproductive characters through time and space. Their integration principally at infrageneric levels is indispensable and has high potential value. The objective of this study is to estimate and understand biodiversity through the detection of morphological-reproductive micromarkers, their intra- and inter-population structure and correlation of genetic, morphological and geographic distances in *Parolinia*, a diploid, exclusively woody Canarian endemic genus (Matthioleae-Brassicaceae) made up of seven similar narrowly distributed species with a similar ecological habitat.

P1398. Natural selection on flower shape and the evolution of zygomorphy in *Erysimum mediohispanicum* (Brassicaceae)J. M. Gomez¹, F. Perfectti², J. P. Martinez Camacho²;¹Dpto de Biología Animal y Ecología, Universidad de Granada, Granada, Spain, ²Dpto de Genética, Universidad de Granada, Granada, Spain.

Paleontological and phylogenetic studies have shown that floral bilateral symmetry (zygomorphy) has evolved independently in several plant groups from ancestors showing radial symmetry (actinomorphy). This evolutionary change is thought to be a consequence of strong selection exerted by specialized pollinators through increasing pollinator preference and improving pollen transfer efficiency. Most approaches have hitherto focused on unraveling the developmental genetics of flower symmetry, but little is known on the adaptive significance of intraspecific flower symmetry variation in natural conditions. Here we provide the first evidence for natural selection favoring zygomorphy in a wild population of *Erysimum mediohispanicum* (Brassicaceae), a plant showing extensive continuous variation in flower symmetry ranging from actinomorphic to zygomorphic flowers. By using geometric

morphometric tools to describe flower shape, we demonstrate that plants bearing bilaterally symmetric flowers were more visited by pollinators and had the highest fitness, measured by the number of seeds surviving to juvenile stages.

P1399. Phylogeny of Burmanniaceae: Preliminary results based on 18S rDNA sequences

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Burmanniaceae are a family of about 130 predominantly myco-heterotrophic annual and perennial herbs. They occur in the tropical and subtropical regions of the Old and the New world. Due to the scarcity of material and the strong vegetative reductions of the mycoheterotrophic species, little is known about their relationships and phylogenetic history. We present the first molecular analysis of the Burmanniaceae based on 18S rDNA sequences. Our sampling includes 40 Burmanniaceae taxa covering six of the family's 13 genera. Analyses using maximum parsimony and Bayesian inference show that Burmanniaceae are polyphyletic: The tribe Thismieae is part of Dioscoreaceae sensu lato while all other Burmanniaceae (tribe Burmannieae) are sister to Dioscoreaceae sensu lato. Within Burmannieae the genera *Burmannia* and *Gymnosiphon* are paraphyletic. The achlorophyllous *Burmannia* species are found throughout the tree, suggesting that the loss of chlorophyll independently evolved more than once within this genus.

P1400. How many species are there? Species delimitation analyses in the genus *Discocactus* (Cactaceae)

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The taxonomy of the genus *Discocactus* has been controversial, with a five-fold difference in the number of taxa recognized by competing classifications. In order to make taxonomic decisions on the specific and infraspecific rank of taxa occurring in the state of Bahia, Brazil, the variation of 22 quantitative morphological characters was analysed for 337 individuals from 17 populations, covering most of the taxa described for the state. We employed the character-based species delimitation method of Davis & Nixon (PAA - Population Aggregation Analysis) and the tree-based species delimitation method of Wiens & Penkrot. The results were compared with those obtained from multivariate and statistical analysis. The methods employed disagree on the number of distinct taxa to be recognized; however, the disagreement is restricted to the level of inclusiveness achieved. We took a conservative view and recognized only the taxa resolved by all methods: *D. bahiensis*, *D. catingicola* and *D. zehntneri*, the latter with two subspecies, *zehntneri* and *boomianus*.

P1401. Taxonomy and diversity of the African non-leucobryoid Calymperaceae species

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In the course of taxonomical revision were established that 49 species occur in Africa and in the neighbouring Madagascar and in the Indian Ocean Islands. The number of species and names which earlier reached more than 180 were reduced with 70 %. The non-leucobryoid Calymperaceae species belong to four Genera in Africa: *Syrrhopodon*, *Chameleion*, *Mitthyridium* and *Calymperes*. The richest Genera is the *Syrrhopodon* comprising 28 species which divided into 39 taxons and have 4 Subgenera and 8 Sections. The Genera *Chameleion* has only one African species - *Ch. cryptocarpus*, and the *Mitthyridium* genus have five species. The Genera *Calymperes* represented in Africa with 15 species which belong to three subgenera. Examining the world distribution of the 49 African species there is established that 42,8 percent of the species are endemic, 24,5 % are paleotropical, 14,3% are pantropical and 14,3 % of the species are African-American disjunct. The list of taxons, the accepted and synonym names of the species and the African distribution are published here.

P1402. Taxonomic revision of *Silene* sect. *Atocion* based on molecular and morphological data

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The Caryophyllaceae genus *Silene* with ca 800 species is hard to diagnose and internal relationships among the species are obscure. Many of the 44 sections recognized by Chowdhuri (1957) in the most recent revision of *Silene* are evidently non-monophyletic. One example is *Silene* sect. *Atocion* Otth, characterized by Chowdhuri primarily on seed morphology, habit and inflorescence type. The subsections *Sedoidae* and *Rubellae* have been demonstrated to belong to different parts of the genus, leaving only eight Mediterranean species in sect. *Atocion*. These species have unclear relations to the rest of *Silene* and within the group. This project is a taxonomic revision based on morphological data, DNA sequences from the nuclear RPB2 and ITS regions, as well as the chloroplast *rps16* region. These data reveal new species delimitations and relationships among the taxa. The pattern of phylogenetic relationships of the taxa and their relationships to the rest of *Silene* are complex and more data are needed to fully understand the phylogenetic history. A possible explanation to the observed complexity is ancient homoploid hybridization among distantly related diploid lineages.

P1403. The Origin of the Hawaiian endemic *Silene* species

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Geographically, one could expect an Asian origin for species endemic to the Hawaiian Islands. Despite this, a number of Hawaiian taxa have been shown to have their closest relatives on the American continents. The endemic Hawaiian *Silene* species were placed in a section with two Japanese *Silene* in the latest global revision of the genus. They have been thought to be the result of two colonizations, one for the shrubby species and one for the remaining herbaceous ones. Our results, based on DNA sequences from the plastid genome, ITS, and the nuclear low copy number gene RPB2 strongly indicate a close relationship between the endemic Hawaiian *Silene* and the American species *S. antirrhina*. This taxon is sister to a monophyletic group consisting of the Hawaiian *Silene*, indicating a single colonization event. *S. antirrhina* is a diploid species that is not related to other native American *Silene*, of which the majority are polyploid and belong to at least three other clades. There is no obvious morphological support for the relationship between the Hawaiian *Silene* and *S. antirrhina*.

P1404. Taxonomy of the *Silene ajanensis* lineage and its participation in the formation of Arctic polyploid *Silene*

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The diploid *Silene ajanensis* lineage has been involved in the formation of both tetraploid and hexaploid Arctic *Silene*. The *S. ajanensis* group comprises about six taxa, distributed in northeast Asia. Traditionally, most of the taxa have been classified in *Lychnis*, based on the presence of five carpels. This condition, however, has evolved several times in *Silene*. We investigate the *S. ajanensis* group in detail, both from a taxonomic standpoint and from multiple gene phylogenies, in order to more fully understand the relationships within this group and its relation to the polyploid taxa. The results will facilitate a more precise hypothesis about timing and geographical location of the allopolyploidization events. Single gene phylogenies from the low copy nuclear RNA polymerase family (RPA2, RPB2, RPD2a, RPD2b) are helpful in resolving this, but also indicate gene duplications independent of allopolyploidization in the *S. ajanensis* lineage. This emphasizes the need to study multiple independent gene phylogenies to discriminate between whole-genome and single gene duplications.

P1405. Reticulate evolution of *Heliosperma* as inferred by nuclear RNA Polymerase Gene Family and chloroplast DNA sequencesB. Frajman^{1,2}, P. Erixon¹, B. Oxelman¹;¹Department of Systematic Botany, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden, ²Biology Department, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia.

Heliosperma Rchb. (= *Ixoca* Rafin., *Silene* L. s.l. p.p), tribe *Sileneae* (Caryophyllaceae), includes about 15 to 20 taxa, most of them being endemic to relatively small areas of the Balkan peninsula. In order to infer the phylogenetic position of *Heliosperma* within *Sileneae* and to examine the relationships within the genus, sequences of a 7kb plastid DNA region as well as homologous nuclear intron regions from the second largest subunit of the RNA polymerase gene family (*RPA2*, *RPB2*, *RPD2a* and *RPD2b*) were used. The phylogenies support a sister position of *Heliosperma* to *Viscaria* and *Atocion*, all forming a monophyletic group with *Petrocoptis* and *Eudianthe*. Incongruence between the plastid and nuclear data indicate an ancient chloroplast capture or lineage sorting of chloroplast genome within *Heliosperma*. Moreover, a homoploid hybrid origin of *H. alpestre* / *H. macranthum* lineage with the *Petrocoptis* as a paternal lineage is indicated.

P1406. A phylogenetic study of *Silene* (Caryophyllaceae) endemic to the Canary IslandsM. E. A. Lundberg¹, A. Santos-Guerra², J. Francisco-Ortega³, B. Oxelman¹;¹Evolutionary Biology Centre, Uppsala, Sweden, ²Jardín Botánico, Puerto de la Cruz, Canary Islands, ³Department of Biological Sciences, Florida International University, FL, United States.

There are seven taxa of *Silene* (Caryophyllaceae) endemic to the Canary Islands. All of them are red listed and endangered. Taxonomically, they have been considered to belong to the section *Siphonomorpha* or more precisely in the *S. italica* group. We have inferred the phylogenetic relationships among the endemic taxa, their taxonomic position within the genus, as well as their probable geographic origin. For this, we analyzed three different DNA sequence regions, the rps16 intron from the chloroplast genome and the nuclear *RPA2* and *RPB2*, both belonging to the low copy number RNA polymerase gene family. The results of the phylogenetic analysis suggest a single origin of the endemic *Silene* found on the Canary Islands. The nuclear data support a sister-group relation to *S. nutans*, whereas the plastid sequences support a sistergroup relationship with a larger group of section *Siphonomorpha* taxa, including *S. nutans*. A possible explanation to this pattern is introgression from the *S. nutans* lineage to the ancestor of the Canarian taxa.

P1407. The morphological characterization of the group *Dianthus brachyanthus*A. L. Crespi¹, S. Bernardos², F. Amich², C. P. Fernandes¹, A. S. Castro¹;¹Herbarium, Botanical Garden, CETAV, Vila Real, Portugal,²Department of Botany, University of Salamanca, Salamanca, Spain.

Four species of the group *Dianthus brachyanthus* (*D. brachyanthus* subsp. *brachyanthus*, *D. brachyanthus* subsp. *hispanicus*, *D. langeanus* and *D. gredensis*) are morphologically analysed. Thirty characters were selected for 210 specimens with occurrence in the Iberian Peninsula at whole. The morphological characterization through the groups of correlation, deduced from the first three factors obtained in the PCA, is depicted as a very useful tool to describe the phenotypical tendencies. The results obtained for the morphological expression of the individuals are correlated with the distribution of the taxa. Two morphological tendencies are detected, one of them for the occidental taxa and the other for the oriental one. The characters related with the morphological variation of the flowers are the most discriminant for the four taxa. Based on the results here exposed, new taxonomical combinations are proposed for these species.

P1408. Biogeography of the afroalpine genus *Uebelinia* Hochst. (Caryophyllaceae) inferred from chloroplast and low-copy nuclear DNA sequencesM. Popp¹, A. Gizaw^{1,2}, S. Nemomissa², C. Brochmann¹;¹National Centre for Biosystematics, Natural History Museum, University of Oslo, Oslo, Norway, ²Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.

The present-day afroalpine plants, which occur in the scattered high mountains of East Africa and Ethiopia, have their closest relatives in other remote mountains, even in Europe and Asia. Recent molecular studies have shown that the primarily afroalpine/afromontane genus *Uebelinia* Hochst. (Caryophyllaceae) is nested within *Lychnis* L., thus representing a southwards extension of a primarily northern plant group. The most recent revisions of *Uebelinia* recognize eight taxa at the species or subspecies level. The group has a discontinuous distribution with the highest number of taxa in Ethiopia, and it also occurs in the mountains of Kenya, Tanzania, Uganda, Rwanda, Burundi, Zaire, Cameroon, and Nigeria. Several taxa have very narrow distributions within single mountain systems. Here we use non-coding chloroplast DNA sequences and intron sequences from a low-copy nuclear RNA polymerase gene family, possibly also AFLP markers, to study the biogeographic history of *Uebelinia*. We also test the monophyly of *Uebelinia* and infer the phylogenetic relationships within the group.

P1409. The family Commelinaceae in Southern Peninsular India

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Commelinaceae with about 41 genera and 640 species world over is distributed mostly in the tropical and warm temperate regions. Peninsular India and the foothills of the Himalayas to Thailand and southwestern China are considered as one of the major center of diversity for Commelinaceae. In southern peninsular India the family is represented by 13 genera and 63 species. Among them 17 species are endemic and many are facing serious threat of extinction. The major genera in southern peninsular India are *Murdannia* (21species), *Commelina* (17species) and *Cyanotis* (11species). *Belosynapsis*, *Amischophacelus*, *Dictyospermum*, are represented by 2 species each, while *Amischotolype*, *Callisia*, *Floscopa*, *Polliia*, *Rhoeo*, *Rhophalephora*, *Setcreasea*, *Zebrina*, are with one species each. *Belosynapsis vivipara*, *B. kewensis*, *Commelina indehiscence*, *C. hirsuta*, *C. tricolor*, *C. wightii*, *Dictyospermum ovalifolium* and *Murdannia fadeniana* and *M. lanceolata* are extremely rare. The family is studied extensively during the past three years. The present paper discusses the diversity, distribution, taxonomy, and endemism of southern peninsular Indian Commelinaceae.

P1410. A Combined Molecular Phylogenetic Analysis of Commelinaceae; Evidence from *rbclL*, *ndhF*, and 26S Nuclear Ribosomal SequencesT. M. Evans¹, C. R. Hardy², R. B. Faden³, R. F. Bode¹;¹Hope College, Holland, MI, United States, ²Millersville University, Millersville, PA, United States, ³Smithsonian Institution, Washington, DC, United States.

Significant progress has been made in recent years toward a comprehensive understanding of phylogenetic relationships within Commelinaceae. Recent developmental and molecular phylogenetic studies have shed light upon the evolution of the family at the generic level or within specific subgroups within the family. These studies, however, have generally included either broad sampling, with low taxon density, or a more intensive focus on a small group of species. In this study, a combined phylogenetic analysis of three molecular data sets (*rbclL*, *ndhF*, and 26S nuclear ribosomal sequences) is presented. Several new genera have been added, including *Pseudoparis*, *Streptolirion*, *Porandra*, *Tricarpelema*, and *Dictyospermum*. Major conclusions of the analysis include: 1) *Cartonema* is sister to the rest of the family, as supported by previous studies; 2) *Palisota* is sister to a clade containing the remainder of tribe Tradescantieae and tribe Commelineae; 3) subtribes Coleotrypinae, Cyanotinae, Streptoliriinae, and possibly Dichorisandrinae are monophyletic; and 4) subtribe Thyrsantheminae is paraphyletic.

P1411. Phylogeny and plastid genome evolution in the parasitic genus *Cuscuta* (Convolvulaceae)S. Stefanovic¹, R. G. Olmstead²;¹University of Toronto at Mississauga, Mississauga, ON, Canada,²University of Washington, Seattle, WA, United States.

Previous findings on plastid (pt) genome structural rearrangements in *Cuscuta* were attributed to its parasitic life style, but without rigorous phylogenetic framework. Long PCR approach was used to sequence nearly entire pt genome from poorly investigated

subgenus *Grammica* in order to determine its size, structure, gene content, and synteny. This pt genome is compared to that of tobacco and corresponding regions from taxa in the other *Cuscuta* subgenera. When all known pt genome rearrangements in Convolvulaceae are considered in a molecular phylogenetic framework three categories of rearrangements in *Cuscuta* are revealed: plesiomorphic, autapomorphic, and synapomorphic. Many of the changes in *Cuscuta*, previously attributed to its parasitic mode of life are better explained either as plesiomorphic conditions within the family, i.e., conditions shared with the rest of the Convolvulaceae, or in most cases, autapomorphies of particular *Cuscuta* taxa, not shared with the rest of the species in the genus. The synapomorphic rearrangements are most likely to correlate with the parasitic lifestyle, because they represent changes found in *Cuscuta* exclusively.

P1412. Visual key for taxa identification on genus *Cupressus*

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Genus *Cupressus* includes species spread in the temperate-warm zones of the northern hemisphere (Mediterranean basin, N-W and central America, Himalayan region and China).

Systematic position of the various taxa within the genus *Cupressus* is not univocal and is frequently reassessed either at species level or subspecies. In the last seventy years a number of taxonomic revisions of the genus have been reported by some Authors. The developed identification keys are all based on morphological characters. Nevertheless morphological characters are greatly affected by a series of anthropogenic factors including, frequently, cuttings and livestock grazing. So, descriptions developed so far are not robust for all the species and, consequently, not always useful too. Identification of the Cypress species in a few instances is difficult and particularly unclear is the systematic position of the Asian species because their native areas are not clearly defined yet. The objective of the present work is to draw up an explanatory visual key for *Cupressus* species based on colour pictures made either in their native areas or on trees of proved origin maintained ex-situ.

P1413. The amino acid composition of seeds in systematics of Cycadales

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The amino acid composition of seeds of 21 species from 7 genera, belonging to 3 families of Cycadales order, - Cycadaceae, Zamiaceae and Stangeriaceae were investigated. It has been shown that all representatives of this order have a similar amino acid composition. Some genera, such as *Dioon* and *Zamia* are very alike in their amino acid composition. *Stangeria eriopus* from monotypic family Stangeriaceae does not differ significantly in any of the amino acids from all the other representatives of cycads. All the investigated species of *Macrozamia* have a similar amino acid composition. *Lepidozamia* differs from *Encephalartos* and *Macrozamia* in lower content of phenyl alanine and leucine and in higher content of arginine and alanine. The difference in the content of some of the amino acids, mainly proline and arginine is marked for species of *Cycas* and *Encephalartos*. It should be noted that the amino acid composition as a systematic feature can be probably used at the generic level, especially while distinguishing homogeneous genera from heterogeneous one as well as when considering taxonomic problems at the level of tribes, subfamilies and close families.

P1414. Study on the natural classification of the wild species of Chinese woody peony

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In 1804 England scientist Henry C. Andrews named and published the first tree peony species "Paeonia suffruticosa Andrews". Andrews' original description of *Paeonia suffruticosa* was based upon a cultivated plant. The plant with very double magenta flowers was planted in England from China. Hereafter, England Peony expert S. G. Haw and China specialist Hong Deyuan ignored the "type tree" of Andrews' *Paeonia suffruticosa* was a cultivar of tree peony. They mistook it as a

"species" or so-called "Paeonia suffruticosa Complex". Thereby, they treated some wild woody peony (very good species!) as the infraspecific taxa of *Paeonia suffruticosa* Andrews.

It is affirmed that *Paeonia suffruticosa* Andrews only includes the cultivars of tree peony and should be strictly separated from any wild species, e.g., *Paeonia rockii* (S. G. Haw et Lauener) T. Hong et J. J. Li in Bull. Bot. Res (Harbin) 12: 223, 1992 [*Paeonia suffruticosa* Andrews subsp. *rockii* S. G. Haw et Lauener in Edinb. J. Bot. 47(3): 279-1990]. The so-called "Paeonia suffruticosa Complex" is an artificially "mixed bag" including cultivars and some wild species of tree peony.

P1415. Revision of the Genus *Knautia* L. (Dipsacaceae) in the Italian Alps

J. Magos Brehm^{1,2}, C. Bonomi³, A. Bonazza³, F. Prosser⁴, N. Macted¹;
¹University of Birmingham, Birmingham, United Kingdom, ²Museu Nacional de História Natural, Jardim Botânico, Lisbon, Portugal, ³Museo Tridentino di Scienze Naturali, Trento, Italy, ⁴Museo Civico di Rovereto, Rovereto, Italy.

Knautia L., a Central European and Mediterranean genus shows high intrinsic taxonomic complexity and a centre of diversity in the Alps. The species are polymorphic and closely related, making identification difficult. The genus is subjected to taxonomic erosion with three species occurring in this region that are listed as threatened under the IUCN Red List categories. As the genus has had limited previous taxonomic study, urgent revision is required to facilitate conservation actions.

Knautia specimens representing *K. arvensis*, *K. longifolia*, *K. baldensis*, *K. illyrica*, *K. purpurea*, *K. persicina*, *K. velutina* species with their provenance in the Trentino region (SE Italian Alps) were loaned from ROV, K, BM and RGN. They were scored for 103 morphological characters for over 100 specimens. The morphometric analysis of the data includes a cluster and a principal coordinates analysis, presenting a revised classification of *Knautia* species found in the Trentino region and thus providing easily usable identification aids for field workers. This will facilitate the evaluation of the species threatened status and the development of regional conservation actions.

P1416. Chloroplast DNA variation in Indonesian Dipterocarpaceae

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Institute of Forest Genetics and Forest Tree Breeding, Göttingen, Germany.

Dipterocarpaceae (subfamily Dipterocarpoideae) dominate tropical rainforests in Southeast Asia. In order to construct a molecular phylogeny of Indonesian dipterocarps we performed PCR-RFLP of the chloroplast regions *rbcl*, *petB*, *psbA*, *psaA*, and *trnLF* with 7 restriction enzymes including 58 species from 9 genera. A total of 104 restriction site polymorphisms and 12 length polymorphisms were detected. In the strict consensus tree with *Monotes kerstingii* as outgroup Indonesian Dipterocarpaceae were divided into two major clades. One clade consisted of *Upuna*, *Cotylelobium*, *Anisoptera*, *Vatica*, *Dipterocarpus* (tribe Dipterocarpeae) and *Dryobalanops* in a basal position (tribe Shoreae). Tribe Dipterocarpeae is monophyletic, tribe Shoreae is polyphyletic since *Dryobalanops* is a sister group to tribe Dipterocarpeae. In the neighbor-joining tree the position of *Dryobalanops* is not resolved. Within genus *Shorea* phylogenetic relationships are poorly resolved and partly in contrast to the classification in botanical sections according to morphological characters. Analysis of additional nuclear genes may help to resolve the relationships within *Shorea*.

P1417. Geographic differentiation within *Shorea leprosula* and *Shorea parvifolia* (Dipterocarpaceae) as revealed by amplified fragment length polymorphism (AFLP) markers

C. Cao, R. Finkeldey, O. Gailing;
Institute of Forest Genetics and Forest Tree Breeding, Göttingen, Germany.

Shorea leprosula and *Shorea parvifolia* are widely distributed in Indonesia and constitute economically and ecologically important species. We analysed 6 populations of *Shorea parvifolia* and 7 populations of *Shorea leprosula* from a total of 6 locations in Sumatra and Borneo and one plantation at 56 well scorable AFLP markers. Cluster Analysis (NTSYS pc2) revealed a clear differentiation between species. A high differentiation between geographic origins could be observed for *Shorea parvifolia* ($G_{st} = 0.31$) and *Shorea leprosula* ($G_{st} = 0.24$). For both species, AFLP

markers with very strong differentiation between geographic regions could be found. For *Shorea leprosula*, we detected one "diagnostic" marker that differentiated between origins from Sumatra and Borneo with a frequency difference of 96%. For two additional populations of both species, specific "private" AFLP markers could be detected. We want to characterize these markers more closely for wood certification purposes.

P1418. Heterospermy in some Ericaceae

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Heterospermy is widely presented in plant world and is inherent both for wild and cultured forms. It manifests firstly in seed size, herewith the sizes of embryo cotyledons and hypocotyl, endosperm, seed mass could change. In the fruits of studied representatives of the Ericaceae (*Andromeda polifolia*, *Calluna vulgaris*, *Ledum palustris*, *Chamaedaphne calyculata*, *Oxycoccus quadripetalus*, *Vaccinium myrtillus*, *V. uliginosum*, *V. vitis-idaea*), collected during dissemination, the seeds varied in sizes, morphology and anatomical structure. Large seeds had, as a rule, normally developed embryo, endosperm and seed coat; the seeds of intermediate sizes usually failed to contain the embryo, and the endosperm either was poor or lacked; small seeds were presented mainly by exotesta, sometimes by the remnants of middle integument layers. Sometimes seeds, except small ones, contained underdeveloped embryos. The ratio of the large, middle and small seeds in the fruit for Leningradskaya region strongly varies. The most high quantity of large seeds has *O. quadripetalus* (about 60%), while in *V. myrtillus*, *V. uliginosum*, *V. vitis-idaea* their amount composes only 30%.

P1419. Molecules and morphology: phylogeny and species delimitation in the genus *Escallonia* (Escalloniaceae)

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Escallonia (Escalloniaceae, Euasterid II-unplaced) includes about 39 species; these are herbs (as *Valdivia*) to trees found in the Andes from Tierra del Fuego to the mountains of Costa Rica, the south Atlantic Forest in Brazil, Juan Fernández Island and, remarkably, Réunion in the Indian Ocean (as *Forgesia*). This genus presents a complex pattern of morphological variation that has led to disagreement over the number of species and uncertainty over its phylogeny. This study will re-evaluate species limits in *Escallonia* using a combination of molecular systematics, morphometrics and natural history. Gene genealogies allow recognizing species as individual segments of population lineages connected by common history. While independent regions with high levels of DNA sequence variation will generate hypotheses of relationship, morphometric analyses will indicate the potential correlation of phenotypic variation at different eco-geographic scales. The results of the different analyses will be compared and, if appropriate, combined to illuminate species limits. The phylogeny will also be used to clarify the perplexing biogeography of this genus.

P1420. "Escallonian Alliance" - an attempt to undo a taxonomic knot with the help of comparative carpology

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Most genera of a compound group usually referred to as "Escallonian Alliance" were originally placed in the subfamily *Escallonioideae* (*Saxifragaceae*), then in the *Escalloniaceae*, but the taxon showed high polymorphism and needed a thorough revision. Currently these genera are distributed between more than 10 families (often monotypic) within *Hydrangeales*, *Saxifragales*, *Celastrales* or even *Cornales*.

Our investigation revealed numerous carpological distinctions between the representatives of this group. The main traits that carpological classifications are based upon (ovary position, number of seeds and carpels, pericarp consistence, dehiscence type) appeared to vary significantly within the group; anatomical differences were even greater. Only a few genera showed resemblance in fruit anatomy (*Carpodetus* and *Abrophyllum* with baccate fruits, *Quintinia* and *Itea* with capsules). Therefore, placing most of the genera into monotypic families seems to be the best solution for "Escallonian Alliance", and the position of some of them (*Corokiaceae*, *Brexiaceae*) is still to be determined.

P1421. Histological studies on cyathia of some *Euphorbia* species

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University of Pécs, Pécs, Hungary.

Euphorbia species have a special inflorescence namely cyathia: it contains a central female flower surrounded by 5 male flowers, 4-5 nectaries and bractea. We studied the histology of the cyathia, with special emphasis on the nectaries in some *Euphorbia* species. Nectaries have cuticle, epiderm, glandular tissue and parenchyma in all species. The xylem vessels have spiral secondary cell wall thickenings. The nectary cuticle of *E. amygdaloides* and *E. palustris* is thin, that of *E. cyparissias*, *E. esula*, *E. myrsinites* and *E. virgata* living at xerotherm area is thicker. Nectary of *E. palustris* has the highest epidermal cells and the largest number of cell rows of glandular tissue (6-7) which varied from 1 to 4 in the other plants. Cells of glandular tissue are isodiametric in all species except of *E. cyparissias*. Biggest cells of glandular tissue were detected in the nectary of *E. virgata*. The histological structure of the nectary was specific for the studied plant species.

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P1423. The Brazilian Caatinga: phytogeographical patterns inferred from distribution data of the Leguminosae

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The Caatinga represents the largest and most isolated of the South American dry forests, occupying an area of almost 900,000 km² in the semiarid region of north-eastern Brazil. Leguminosae is the best represented family in the Caatinga, with 292 species in 77 genera, and comprises almost one third of the total plant diversity there. The geographical distribution patterns of its taxa define seven major centres of endemism. Similarity analysis combined with these patterns reveal two major floristic groups with distinct composition and phenological traits. One group occupies c. 70 % of a mostly continuous surface of soils primarily derived from crystalline basement rocks. The other group occurs on disjunct and sandy sedimentary surfaces. The data supports a scenario wherein the sedimentary areas became dissected during a huge process of pediplanation during the Tertiary period, which promoted both the vicariance of its flora and opened the way to the expansion of the flora related to the seasonally dry tropical forests.

P1424. Research of different of Nitrogen and Phosphorous on the yield of Sainfoin in dry land conditions

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Sainfoin (*Onobrychis sativa*) is a soft forage plant with high protein content. This plant has some characteristics such assistance to unsuitable environmental conditions like low fertility of soil and intensive cold of winter, resistance to drought in dry land farming with acceptable yield, resistance to overgrazing in rang lands and direct grazing capability without causing the blowing.

To study the effect of various levels of Nitrogen and Phosphorous fertilizer on the forage yield of Sainfoin of experimental design was carried out as the randomized complete block design with four replication and two factors. Nitrogen fertilizer in three levels and the other phosphorous fertilizer in four levels. The main purpose of

this experiment was to introduce scientific strategies to sure of the daneerlees use of chemical fertilizers to produce acceptable yield with sustainable environment conservation purpose.

In this study, dry matter yields ,wet weight yield ,and shoot height were measured and compared.

In spite of shoot height above mentioned characteristics was significantly affected by various Levels of Nitrogen and Phosphorous fertilizers.

P1425. Lower Miocene Leguminosae flowers from the amber of Simojovel, Chiapas, México

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Leguminosae abundance/diversity in Mexican extant vegetation resembles that of the Tertiary flora of the region. They are collected in most Eocene-Recent localities bearing flowering plants. New amber samples contain flowers comparable with those of legume plants. An inflorescence with 8 pentamerous, actinomorphic, perfect flowers, each with ca. 10 floral bracts, gamosepalous calyx with 5 small pubescent and striated lobes, 5 lobed thin gamopetalous corolla, abundant free stamens (ca. 50) with pedicelated glands on dorsifixed anthers, and ovary with thick large apical style, resembles those of *Acacia* (Acacieae) or *Albizia* (Inegeae). A second flower resembles that of *Hymenaea mexicana* (Detarieae) in being actinomorphic and perfect, pubescent in pedicel and receptacle, having 3 elliptic free sepals, free stamens with dorsifixed anther, and ovary inserted on a disc with seeds in axilar position. These flowers confirm that Leguminosae were established some 23.5 mybp in southern Mexico.

P1426. *Astragalus* sect. *Inceni* (Fabaceae) in Iran

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sect. *Inceni* (subgen. *Cercidothrix*) belongs to bifurcate haired group *Astragali*. Iran is the center of diversity of the section, with cf 50 species of which about 35 are endemic. New findings are given for the *Astragalus* sect. *Inceni*, which occurs within the area of flora of Iran. The seed surface sculpturing of *Astragalus* have been studied. The basic pattern of the seed testa in Iranian *Astragalus* particularly between sections is remarkably heterogeneous. Variation arises from differences in both size and shape of the seed. The shape of the seed is oblong, square or reniform. Its color is light or dull brown. The surface of the seed is usually rough (observable with SEM). Results showed micromorphological diversity and taxonomic value of this feature.

Five seed coat patterns were found: A) bilayer-reticulate, B) monolayer-reticulate C) reticulate-verucate, D) rugulate and E) foveo-rugulate. The evolution trends of testa micromorphology show phylogenetic relationships. As fine reticulate pattern existed commonly, the pattern is regarded as the most primitive type. Other types are derivatives from the basic reticulate pattern.

P1427. Biosystematical study on the genus *Lathyrus* (Fabaceae) in Iran

R. Karamian¹, M. Ranjbar¹, V. Niknam², M. Hedyelu¹;

¹Bu-Ali-Sina, Hamadan, Islamic Republic of Iran, ²University of Tehran, Tehran, Islamic Republic of Iran.

Morphological study were performed on 23 species of Iranian *Lathyrus* using 38 characters. Also micromorphological study on seed surface were done by SEM. Seed storage proteins of seeds were studied by SDS-PAGE gel electrophoresis. Flavonoid compounds in glycosyle ester forms were extracted from leaves and separated by TLC method. Obtained dendrogram from morphological studies by NTSYS software shows that there are 5 sections in the genus that confirm its current classification in flora of Iran. Results from the micromorphological and flavonoid compound studies confirm above results and show that *L. cicera* and *L. sativus* are closely related. Also *L. pratensis* and *L. Laxiflorus* are placed in the unique section and *L. Boissieri*, *L. cyaneus* and *L. brachypterns* are placed in another section. Results from electrophoretic study do not confirm above results compeletly and are not suitable for classification of the genus.

P1428. Taxonomic significance of anatomical characters and pollen morphology in *Astragalus* sect. *Onobrychoidei* in Iran

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¹Bu-Ali-Sina, Hamadan, Islamic Republic of Iran, ²Isfahan University, Isfahan, Islamic Republic of Iran.

Astragalus L. with more than 840 species, is the largest genus in the flora of Iran. It is the most problematic group in legume systematic. *Astragalus* sect. *Onobrychoidei* belongs to the bifurcate haired group *Astragali*. Iran with more than 25 species is one of the main centers of diversity of the section. The pollen surface sculpturing of the species of the section has been studied. Two basic types of the pollen surface were observed in these Iranian *Onobrychoidei*. Also in order to evaluate the internal structure in the classification of the section, 31 anatomical characters were studied. The results showed that the stem and peduncle have different shapes (e.g. stellate, orbicular and sometimes trapezoid) in different taxa. Furthermore, epidermal features, type of collenchyma cells, type of vascular bundles and the habit of the stem have important value in distinguishing the species.

P1429. Species Richness and Distribution Patterns of the Colombian Species of *Inga* (Leguminosae: Mimosoideae: Ingeae)

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Inga is one of the largest neotropical genera of Leguminosae (c. 300 species). In the process of preparation of a taxonomic revision of *Inga* for Colombia, 95 species were found, including 29 new records for the country. The 95 species represent 11 of the 14 sections recognized by Pennington (1997). The genus is widely distributed in the country and has 3 centers of species diversity: the Amazon, the Andes and the Pacific region (62, 58 and 48 species respectively). In Colombia, the species of *Inga* grow in a wide range of habitats such as gallery forests, wet/very wet tropical forest, pluvial tropical forests, forests on periodically flooded lands, grass pastures and forest on terra firme in the Amazon region. The genus also exhibits wide altitudinal distribution, ranging from sea level to 2900 m. Most species are widely distributed altitudinally, while only a few show restricted altitudinal distribution patterns.

P1430. A contribution to the systematics of annual species of *Astragalus* (Fabaceae) in the Old World

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Sixty annual species of *Astragalus* were analyzed for hair micromorphology using SEM and floral morphology. Characters from hair micromorphology, flower morphology and some vegetative characteristics of the plants have been used in a cladistic analysis of annual *Astragalus*. The analysis resulted in 12 trees. The strict consensus tree using morphological characters is in several aspects similar to the cladogram presented recently based on ITS sequences. In both cladograms most of the species attributed to sects. *Hispiduli*, *Platyglottis* and *Sesamei* represent the most derived group and form a monophyletic clade. The monotypic sections *Ophiocarpus*, *Dipelta* and *Mirae* cluster within the same clade. The most important disagreement between the cladograms is regarding sections *Bucerates*, *Oxyglottis* and a part of sect. *Annulares*. Morphological characters correlating the species attributed to these groups are plesiomorphic and can only characterize paraphyletic groups. Based on available data from molecular systematics and cladistic analysis of morphological characters, circumscription of several sections in *Astragalus* should be changed.

P1431. Spine anatomy and its systematic application in *Astragalus* sect. *Rhacophorus* s. l. (Fabaceae) in Iran

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¹Central Herbarium of Tehran University, Tehran, Islamic Republic of Iran, ²Institut für Systematische Botanik der LMU, Munich, Germany.

Cross sections of petioles in 35 species mainly belonging to *Astragalus* sect. *Rhacophorus* were studied for insights into circumscription of natural groups within the section and reliable characters in delimiting polymorphic species. The following characters were determined to be informative: number of parenchymatous cell layers in pith, diameter of median sclerenchymatous vascular bundle sheath and diameter of the cross-section as whole. *Astragalus amblolepis*, *A. andalanicus*, *A. floccosus*, *A. meyeri*, *A. paraliipomenus*, *A. pycnocephalus* and *A. stenolepis* are characterized by large pith and relatively large sclerenchymatous bundles. Most of these species belong to sect. *Rhacophorus* s. str., comprising large-flowered species as circumscribed by some taxonomists, but few species of sects. *Stenonychium* and *Platonychium* also show these features. Our results show that spine anatomy is not useful for circumscribing sections in spiny *Astragalus*, but applicable in delimiting small natural groups and circumscribing species.

P1432. Testa topography & Seed Coat Anatomy of the Genus *Alysicarpus* Desv.

D. S. Pokle, A. S. Dhabe;
Vivekanand College, Aurangabad, India.

Since last 10 years revision of genus *Alysicarpus* for India was undertaken. It was felt that more exomorphic studies are insufficient for taxa delimitations. Hence other parameters were exploited. One of these parameters is seed testa topography and seed coat anatomy.

Seeds of 17 taxa were examined by SEM. Two note worthy features were noted. First, duplex testa topography was found in 9 taxa. Secondly, 15 taxa show multirugulate and faveolate pattern. According to Larsten, these features are prevalent in tribe *Hydesareae*. This feature is of evolutionary significance.

Seed coat anatomical studies of 24 taxa were undertaken. Macroscleireids, Osteosclereids, Parenchyma and Endosperm are unique in each taxon at even variety & form level. These anatomical features are taxon specific. This feature is most reliable for taxa delimitation.

P1433. Phytochemical Studies on the Genus *Alysicarpus* Desv.

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Dr.B.A.M.University, Aurangabad, India.

Alysicarpus Desv. is a tiny herbageous genus of fabaceae-Desmodeae. During the course of investigations, phytochemical studies were undertaken on Alkaloids, Iridoids, Tannins, Saponins & phenolics, which are taxonomically significant. In all these alkaloids are of not much importance taxonomically. Overall results of phytochemical analysis of 24 taxa (13 species, 7 varieties & 4 forms) are presented in the paper. On the basis of phytochemical analysis, it was revealed that a dimorphic form of *A.monilifer*, erect & trifoliolate forms of *A.scariosus* should be raised into new species (sp.novo). *A.vaginalis* var.*nummularifolius* be raised to species level & *A.hamosus* trifoliolate form be raised to variety level. In general *A.bubleurifolius* var.*gracilis* is most primitive and *A.luteo-vexillatus* & *A.monilifer* (dimorphic form) are most advanced among the genus.

The analysis for fodder nutrient proved that the almost all species are nutritionally compatible to *Medicago sativa* even in wild state.

P1434. Evolution of the series *Microphyllae* (Subgen. *Acanthadesmia*, *Adesmia*, Fabaceae) in the Andean ranges: A biogeographic perspective

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This study explores a preliminary hypothesis about the evolution of the series *Microphyllae* (Subgen. *Acanthadesmia*, genus *Adesmia*, Fabaceae), comprising about 33 species. *Microphyllae* is distributed exclusively in the Andean region of South America, from Peru (13°W) to Argentina (40°W) representing the total distribution of the subgenus *Acanthadesmia*. The biogeographic history of this series is unclear, and current knowledge does not enable a clear understanding of the evolutionary history of the genus.

We carried out a track analysis based on the distributional patterns of 22 species of this series. Five generalized tracks were found

and two nodes were identified. Parsimony analysis of endemism of the individual track split the total area in two (northern and southern) sub-areas. Our result suggest a recent differentiation resulting from isolation of populations along the Andean cordillera, i.e. vicariance events fragmented the distribution and caused subsequently differentiation.

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P1435. Study of the Flora of Cicer (Papilionaceae) in Iran

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The genus *Cicer* which belongs to the tribe Viciae of Papilionaceae is very well known by the globally important cultivated species *Cicer arietinum* L. other than the cultivated species. It also comprises forty two (42) species in the world (among two subgenera) from which ten (10) species grow in Iran. The aim of this study is providing the flora of *Cicer* including description of taxa, the keys for determination and the geographical distribution of Iranian species. For this purpose a classical systematic study was performed based on morphological (vegetative as well as reproductive) characters. According to these studies the taxonomic status of *Cicer kermanense* was changed from species to subspecies and *Cicer bijugum* was introduced as a new record for the flora of Iran.

P1436. Study of the Flora of Cicer (Papilionaceae) in Iran.

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The genus *Cicer* which belongs to the tribe Viciae of Papilionaceae is best known by the globally important cultivated species *C. arietinum* L. other than the cultivated species. It also comprises 43 species, from which 10 species grow in Iran. The aim of this study is providing the flora of *Cicer* including description of taxa, keys for determination and geographical distribution of Iran species. For this purpose classical systematic study was performed based on morphological (vegetative and reproductive) characters. The numerical taxonomy was done by cluster analysis. As a result, Leaflet numbers and peduncle length have maximum variance and leaflet form (leaf or spine form) has minimum amount of variance. According to these studies the taxonomic status of *C. kermanense* was changed from species to subspecies and *C. bijugum* was introduced as a new record for the flora of Iran.

Keywords: *Cicer* species, Papilionaceae, *Cicer arietinum*, classical systematic.

P1437. Fagaceous fossils from the Eocene Buchanan Lake Formation, Axel Heiberg Island, Canadian High Arctic

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Fagaceous fossils represented by pollen, leaves, and cupule/nuts were recovered from middle Eocene Buchanan Lake Formation, Axel Heiberg Island, Canadian High Arctic. They are assigned to three extant genera, viz. *Castanea*, *Fagus*, and *Quercus*, and one unassignable taxon (Fagoideae gen. et sp. indet.). The first two genera are only represented by pollen, among which one pollen type of *Castanea* and three pollen types of *Fagus* were recognized, while the occurrence of *Quercus* was supported by both pollen and leaves. On the basis of leaf and pollen morphology, the oak fossils are assigned to two sections of subgenus *Quercus*, viz. sect. *Lobatae* and sect. *Quercus*. The unassignable taxon represents the cupule and nuts, displaying characters not in any extant fagaceous taxon, thus probably representing an extinct member of early Fagaceae lineage. All the fossils might be deciduous in nature, which is consistent with paleoclimate and paleofloristics, reconstructed from other evidence. The study shows that the *Fagus* pollen are probably one of the earliest record and the red and white oaks would be differentiated as early as in the middle Eocene.

P1438. Wood anatomy and chemical identification of oak (*Quercus*)

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The wood anatomy of *Quercus* is rather homogeneous, but typically 3 groups of species are recognized: ring porous white oaks, ring porous red oaks and live oaks, which are diffuse porous. In addition to porosity, features used to distinguish the groups are ray height and latewood vessel arrangement. In temperate regions white and red oaks generally follow taxonomic classifications, but subtropical and tropical regions only have live oaks. The phytochemistry of oaks generally revolves around tannin chemistry. Although tannins are present in all oaks, there is a distinct difference in the tannins or precursors between white and red oaks. A 10 % solution of sodium nitrite applied to the transverse surface of oak heartwood clearly distinguishes red and white oaks. White oaks show a positive reaction turning a dark blue or black and the red oaks are negative showing an orange color. This test was performed on all the specimens in our collections and few exceptions were noted.

P1439. Genetic variability of *Quercus faginea* and *Q. broteroi* in Iberian populations

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The genus *Quercus* L. has several taxonomic problems due to its frequent hybridisations and introgressions in the Iberian Peninsula. *Quercus faginea* Lam. was considered as a species divided in two subspecies: *Q. faginea* subsp. *faginea* and *Q. faginea* subsp. *broteroi* (Coutinho) A. Camus. However some authors distinguish them as two species: *Q. faginea* and *Q. broteroi* (Coutinho). Furthermore, these authors indicate two subspecies for *Q. faginea*: *Q. faginea* subsp. *faginea* and *Q. faginea* subsp. *alpestris* (Boiss.) Maire.

After reviewing the morphology of both species, the aim of this work is to find out its biogeographic and genetic variation. Populations of *Q. faginea* and *Q. broteroi* across the Iberian Peninsula were used for this study. Also, other species of genus *Quercus* were included as outgroup. The molecular biology techniques carried out were RFLP and SSR markers. For the first study two pairs of primers from cpDNA were used: trnC-D and trnD-T. The amplified products were digested with Taq I revealing low polymorphism. For this reason, one microsatellite marker from cpDNA (ccmp3) and four from nrDNA (ZAG ½, 15, 36 and 110) were used showing more variability.

P1440. Geometric morphometrics leaf variation in oaks (*Quercus*) of Central-Southern Italy.

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Genus *Quercus* has a problematic taxonomy because of widespread hybridization between the infragenetic taxa. *Q. pubescens*, *Q. virgiliana* and *Q. dalechampii*, dominant trees of mediterranean and temperate forest in Central-Southern Italy, are studied. The pattern of morphological leaf variation was evaluated for evidence of hybridization. Since previous studies suggest that oak leaf morphology is a good indicator of putative hybridization. Geometric morphometrics methods were applied to two data sets of leaf characters from six populations. Landmarks and entire outlines were compared to describe variation in leaf morphology. Similar results were found when differences in shape were analysed by discriminant analyses of shape coordinates (landmarks) and Fourier coefficients (outlines). It appears that there is hybridization involving three taxa studied and the hybridization between *Q. pubescens* and *Q. virgiliana* is greater, in the populations sampled.

P1441. Spatial distribution model of cork oak (*Quercus suber*) in south-western Spain

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A spatial distribution model of cork oak (*Quercus suber*) has been developed for the province of Huelva (south-western Spain). This model has been carried out by means of the available digital cartographic information of cork oak distribution. The topographic data (altitude, slope and orientation) have been obtained from the Digital Terrain Model (20 m). Rainfall, temperature and PET models have been based on data collected from the network weather stations (NIM). Litologic data have been obtained from litologic map of Huelva (1:100.000). The final scale of work is a mesh of points of 100 m of resolution. Each point of this mesh contains the corresponding values of bioclimatic, topographic and litologic variables in a georeferenced data matrix. Applying binary logistic regression analysis to the data matrix, those variables responsible of cork oak distribution have been pointed out. The spatial distribution model has been produced by applying the equation obtained in the regression to spatial analyst software. This model is proposed as a basis of future reforestation plans, especially in more affected areas by forest fires.

P1442. Where does the Italian cork oak (*Quercus suber* L.) come from? A phylogeographic study using chloroplast markers.

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Cork oak is distributed in Western Europe. It is still debating whether the centre of origin and diversification has to be found only in the Iberian Peninsula. In this study we analysed by chloroplast DNA markers over 40 cork oak populations collected within the Italian distribution range, and compared them with populations sampled in the rest of the natural range. The goals were: i) to describe the phylogeographic structure and to investigate the possible origin of the Italian populations; ii) to infer the potential human impact in shaping the chloroplast diversity. Results showed a strong genetic differentiation among populations. Three groups of haplotypes were detected in Italy, suggesting different origins: 1) Northern-central Italy, 2) the Southern-central regions, including Sicily, and 3) Sardinia. Italian populations do not share haplotypes with the other European and African populations, with the exception of Sardinia (and Corsica) which have the same haplotype of Tunisia and Algeria. We speculate about the possible role that ancient Mediterranean civilisations might have played in determining the present day distribution of cork oak.

P1443. On the ecology and distribution of *Quercus macranthera* Fisch. et May.

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Quercus comprises 350-500 species distributed throughout the Northern Hemisphere, as conspicuous members of both temperate deciduous and evergreen forests of North America, Europe and Asia. Oak taxonomy is often complicated, as some taxa are not clearly differentiated and distinguishable or may present large clinal transitions in their intermediate positions.

The purpose of the present study was to assess the ecological and distributional features of *Quercus macranthera* Fisch. et May, an oak species endemic to Caucasus and Asia Minor whose geographic range is not yet well defined, mostly due to scarce data and uncertainties on the ranking value of some populations. We present a comprehensive distributional map of the species, including all (eventual) intra-specific entities. Altitudinal, climatic and vegetation components of the interested areas are reported so that the main ecological requirements of the species are delineated. Collected data allow to support the hypothesis of the Euxinian forests (along the southern shores of the Black Sea) as a glacial refugium, with the Caucasus Mountains probably acting as a significant biological barrier.

P1444. Post-glacial evolutionary trends within the genus *Quercus* in Eurasia and America: Divergence versus genome sharing

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Climatic cycles during the Quaternary had far-reaching influences on distributions, population sizes, genetic diversity and differentiation in *Quercus*. Divergence associated with displacement to multiple glacial refugia has been documented for European and western North American species. Rates of recolonization of vacant habitat following the last glacial retreat have been remarkably rapid for *Quercus* considering seed weight and morphology, so that long-distance dispersal events must have been an important mode of migration. Recently, pollen dispersal followed by introgression has been invoked to explain observed patterns of chloroplast sharing in European and western North American species and this may have contributed to long distance dispersal. The high frequency of hybrids within all sections of the genus *Quercus* from North America and Eurasia has been the source of much taxonomic confusion, but may have major evolutionary consequences. Has pollen dispersal been a general mode of colonization since the last glacial maximum in a genus renowned for hybridization and are hybrids merely a dead end, or are they contributing to evolution in this genus?

P1445. Current status of the genus *Quercus* in the Iberian Peninsula

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In *Flora Iberica* there are twelve species reported. Then two more species have been described, *Q. pauciradiata* and *Q. orocantabrica*, and we consider some taxa at different taxonomic level *Q. rotundifolia*, *Q. broteroi* and *Q. subpyrenaica*. We have noticed that hybrids are very frequent when the parents live together, although the hybridization occurs only in some spots of the sympatric area. The leaf trichomes are very specific, and most species can be identified by means of their leaf trichomes. The hybrids always have the trichome types of both parents. *Q. pauciradiata* is considered critically endangered. For other species, there are populations in the edge of its distribution area as *Q. lusitanica* in Galicia, which is considered threatened by hybridization with other species. As many species are forming forests, and there are several laws at regional level protecting some forest types, the species of *Quercus* are protected by these means. Finally, we offer distribution maps as accurate as possible in order to establish the distribution area for the taxa in which this information was not available, and an identification key.

P1446. Taxonomical , anatomical and palynological study on *Muscari* Mill. species in Iran.

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Muscari Mill. is belong to Scillea tribe and Liliaceae. It has 10 species, 4 species from subgen. *Leopoldia*, 4 species from *Botryanthus* and 2 species from *Pseudomuscari* in Iran. In this research a record is introduced (*M. microstemum*) from subgen. *Botryanthus*. In anatomical study leaves, stems, roots were cut by microtome (12 micron) and stained by saffranin and fast green. Root had spiral vessel and stem had tracheid. Leaf upper and lower mesophyll is palisadic in subgen. *Leopoldia* and circular-elliptical in subgen. *Botryanthus* and circular in subgen. *Pseudomuscari*. In stem is posed epidermis, 2 layers parenchyma, several layers sclerenchyma and parenchyma. For palynological research, pollens were elliptic, monosulcate, assymetric, distal polar, semitectate and reticulate. In subgen. *Leopoldia* and *Pseudomuscari*, furrow is shallow and narrow and in the subgenus this is deep and large. The result is: anatomy and palynology can show differences between subgenus

P1447. Morphometric analysis of Croatian and Slovenian populations of *Iris sibirica* sensu lato (Iridaceae)

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Species *Iris sibirica* L. s. l. in Croatia and Slovenia include populations from lowlands and mountains. The taxonomical status of mountain populations is not clear, although some authors recognize them as a different taxon (*Iris erirrhiza* Pospichal). As populations variability was detected, but until now not researched, we started with morphometric analyses of populations to detect their possible differentiations. A total of 108 specimens from eleven populations in Croatia and Slovenia were collected. Features for morphometry included: stem length, number of leaves, greatest width of the largest and smallest leaf, length of the largest and smallest leaf, length of the spathe, number of flowers, pedicel length, widths and lengths of falls and standards, length of anthers and filaments, ovary length and length of styles. Relationships between populations and individual plants were analysed by methods of univariate and multivariate statistics. On the basis of investigated features there was no separation of mountain from lowlands populations. The results are in favour of the opinion that both types of population belong to the same species.

P1448. The ecological importance of a chemotype polymorphism in *Thymus vulgaris*.

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Thymus vulgaris provides a fascinating example of genetic polymorphism in secondary compound production. In southern France six distinct chemical types (chemotypes) are found. Local populations are mainly dominated by one or two chemotypes creating a striking spatial pattern of chemotype distribution on a very local scale. Our research focus on whether chemotypes show local adaptation to their abiotic and biotic environments and if associated species also show performance variation indicating adaptation to the different chemotypes. Reciprocal transplants of seedlings and adult plants among sites dominated by phenolic and non-phenolic chemotypes were followed over four years. Difference in performance among chemotypes show that phenolic outperform non-phenolic chemotypes in their phenolic populations providing strong evidence for local adaptation. The performance of an associated grass species from sites dominated by either phenolic or non-phenolic chemotypes provides evidence of adaptation to the environmental modifications associated with the local thyme chemotype. Adaptive variation of thyme and its associated plant species are discussed

P1449. Epidermal leaf structures of the species of genus *Micromeria*, section *Pseudomelissa* (Lamiaceae)

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The genus *Micromeria* Bentham includes about 120 species widespread in the Mediterranean region (Doroszenko, 1986). In the flora of Serbia and Montenegro this genus is represented by 10 species, seven of which are endemic (Silic 1979). On the basis of their morphological characteristics the species of the *Micromeria* genus are grouped in three sections (Boissier 1879): *Cymularia*, *Eumicromeria* and *Pseudomelissa*. In this work we are going to describe surface leaf structures of the following species of section *Pseudomelissa*: *M. thymifolia* (Scop.) Fritsch, *M. dalmatica* Bentham, *M. pulegium* (Roche) Bentham and *M. albanica* (Griseb. ex K. Maly) Silic. Features such as: thickness and ornamentation of the cuticle, epidermal cell walls characteristics, number, distribution and type of stomata, and indumentum density (number and size of the nonglandular and glandular peltate and capitate trichomes) represent structural adaptations to a complex of factors present in the habitats populated by the investigated species. These features can be used as additional characters when defining the species of section *Pseudomelissa* genus *Micromeria*.

P1450. Molecular phylogeny of *Conradina* and other scrub mints (Lamiaceae) from the southeastern USA using nuclear and plastid markers: evidence for hybridization in Pleistocene refugia?

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Conradina (Lamiaceae) consists of six allopatric species endemic to the southeastern United States, four of which are federally endangered or threatened. The limits and status of several taxa have been contested based on morphological grounds, and clarification of these limits is necessary for the design and implementation of effective and fiscally responsible protection and management plans. The objectives of this study were to investigate the monophyly of *Conradina* and its relationship to other related mints which are endemic to the southeastern United States, to understand the patterns of diversification in *Conradina*, and to clarify species relationships. A molecular phylogeny was inferred by sequencing regions from the nuclear and plastid genomes from multiple accessions of each species of *Conradina* and multiple individuals from species of *Clinopodium*, *Dicerandra*, *Piloblephis*, *Stachydeoma*, *Monarda*, *Pycnanthemum*, and *Mentha*. The nuclear and plastid phylogenies conflict, which may be due to shared ancestral polymorphism and lineage sorting, or more likely, introgression that occurred very recently or during the Pleistocene.

P1451. A molecular perspective for tribal concepts and generic boundaries in subfamily Nepetoideae (Lamiaceae)

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Among the subfamilies of Lamiaceae Nepetoideae seems to be a well defined monophyletic lineage with three tribes recognised (Elsholtzieae, Ocimeae and Mentheae). Tribal subdivision and generic concepts remain speculative and unclear. We performed comparative sequencing of the *trnK* intron and *trnL-F* region to test relationships within Nepetoideae. Tribes Elsholtzieae and Ocimeae are indicated as subsequent sisters to tribe Mentheae. Subtribe Salviinae is well defined followed by some genera of more or less uncertain affinities. Subtribe Nepetinae is well supported sister to subtribe Menthinae, the members of which are arranged in a four clade polytomy. One clade represents *Satureja* s.str., a second includes genera *Thymus*, *Origanum* and their close relatives. Two sections of *Micromeria* form a third lineage, while one is embedded within a clade chiefly containing *Clinopodium* species. This group colonised the New World probably once starting from Africa. We could confirm tribal but only partially subtribal or generic concepts. *Micromeria* appears polyphyletic and *Clinopodium* as currently circumscribed is paraphyletic to a vast number of genera.

P1452. Biosystematics of tribe Scilleae (Liliaceae) in India: Northern Western Ghats: a likely centre for diversification of tribe Scilleae (Liliaceae) in India

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Tribe Scilleae of the family Liliaceae is represented by three genera viz. *Dipcadi*, *Drimia*, *Scilla* and about 16 species in India. Northern Western Ghats of India (hotspot of plant diversity) harbour 12 species of which 10 are endemic and restricted to the region. Genus *Scilla* is represented by 3 species of which two occur in northern Western Ghats. *Scilla hyacinthiana* is represented by cytotypes with peculiar distribution pattern, morphological variations and blotching patterns of leaf. Out of the 5 Indian species of the genus *Drimia*, 4 are endemic to northern Western Ghats. Both diploids and tetraploids of *D. congesta*, *D. indica* occur in the region with definite pattern of distribution of cytotypes. *Drimia indica* is found almost throughout India but triploid are restricted to coastal regions of India. Genus *Dipcadi* is represented by 8 species in India of which 6 are found in northern Western Ghats. The study suggests northern Western Ghats to be the likely centre for diversification of the tribe Scilleae in India.

P1453. A Comparative Pharmacognostic Investigation of the Leaves of *Aloe schweinfurthii* and *Aloe vera*

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The *Aloe* plant (family: Liliaceae) has been used all over the world for many years for various medicinal and health purposes. Studies on the macro- and micro-morphology of *Aloe schweinfurthii* Baker with reference to those of *Aloe vera* (Linn.) Burm. f., a world acknowledged *Aloes* species, were carried out on the leaf for their comparative identification, authentication, chemo-microscopy, quantitative microscopy and phytochemical profiles that could be used as part of their monographs in the proposed Nigerian Herbal

Pharmacopoeia (NHP). The results showed that both *Aloe* species possess many similarities in morphological characters, with some distinct diagnostic differences in their physical characteristics. For example, the stomata number and stomata indices on the lower and upper surfaces for both species show some similarities and differences. Comparative phytochemical screening of the methanolic extracts of both *Aloe* species revealed the presence of free and combined anthraquinones, starch, flavonoids, steroidal and phenolic compounds.

P1454. Systematic Position of *Lilium akkusianum* R.Gämperle (Liliaceae) Based on Morphological and nrDNA ITS Sequence Data

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Lilium akkusianum R.Gämperle is an endemic species to North-Eastern Turkey. It has a very narrow distribution area composed of only a few populations. It is closely related to another Turkish endemic *L. ciliatum* P.H.Davis, but can be distinguished from it by larger, less recurved flowers and smaller bulbs. These two endemic species differ from the other yellow-flowering Turkish lilies by long-ciliate hairs on their leaf margins and glabrous leaf veins. Distribution of *L. akkusianum* does not overlap with distributions of other Turkish *Lilium* L. species. Phylogenetic analyses based on majority rule of the sequences of the nuclear ribosomal DNA internal transcribed spacer region (ITS) confirmed the close relationship between *L. akkusianum* and *L. ciliatum*. The results of the molecular studies also revealed that there is sufficient molecular divergence between these two species to support their status as separate species.

P1455. Karyological studies of Liliaceae s. l. taxa in Transcarpathian Region (Ukraine)

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In the Transcarpathian Region 35 to 38 wild species (39 to 41 including subspecies) of Liliaceae s. l. (sensu Krause) grow in nature. Cytotaxonomic studies and comparative karyological analysis of about 150 natural populations of these taxa were carried on. It was found that along with stability in some species there is intraindividual and interpopulational variability of the karyotypes according to the level of ploidy, morphology of individual chromosomes, nucleolar-organiser regions of the satellite chromosomes and B-chromosomes. In particular, a considerable B-chromosome polymorphism was observed in *Lilium martagon* L. populations, taxa of genus *Ornithogalum* L., the latter showing the inhibiting influence of B-chromosomes accumulation on the development of individuals.

Cytotypes relationship of Transcarpathian and Balkan populations was stated, as well as their difference from the Central European cytotypes, which indicates the Balkan paths of invasion of these species in Transcarpathia. On the other hand, a number of taxa in their karyotype structure are similar to the cytotypes of Central European populations and differ from the Balkan ones.

P1456. A contribution to the systematic relationships among some European taxa of genus *Lilium*

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In this paper systematic relationships among several European taxa of genus *Lilium* are discussed, with special observations about the morphologically similar taxa belonging to the *L. carnioolicum* group (*L. pyrenaicum*, *L. carnioolicum*, *L. bosniacum* and *L. jankae*) that have historically been a cause of doubt or confusion. Another objective was to analyse the relationships between *L. martagon* and its close relative, a Dalmatian endemic taxa with an unclear taxonomic status, *L. cattaniae*.

Combined analysis of the restriction-site variation of PCR-amplified cpDNA fragments (*trnT-trnL*, *trnL-trnF* and *atpB-rbcL*), mtDNA fragment (*nad4* intron) and nrDNA ITS data was conducted as a first step towards a better differentiation of dubious taxa and to provide the basis for phylogenetic comparisons.

The obtained restriction patterns effectively separated clear taxa, but provided no resolution of closely related taxa. The most systematically valuable was the ITS region which provided separation of clear taxa with all used restriction enzymes.

P1457. Taxonomic revision of the genus *Sticta* (Lobariaceae, Peltigerales) in East Asia

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The macrolichen genus *Sticta* are distributed throughout the world but centered in Southern Hemisphere. East Asian species of *Sticta* were taxonomically revised. Eleven species were recognized to occur, among which a photosymbiodeme was observed only in *S. wrightii* Tuck. Molecular phylogenetic approach was first applied for the photosymbiodeme of *S. wrightii* based on the specimens collected from Yunnan, China. It suggested that the morphologically different photomorphs of this species share a single mycobiont. The characters recognized to be of taxonomic importance include; (1) the cortex structure including the thickness of epicortex and luster, (2) the shape of isidia (cylindrical to coralloid or phyllidiate), (3) the margins of cyphella (thickened or not thickened), (4) the basal anatomy of apothecia (apothecia with a constricted base or a non-constricted base), and (5) the spore shape (acicular to linear or fusiform).

P1458. Five species of Magnoliaceae New to the flora of Thailand

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The revision of Magnoliaceae in Thailand was reported by King in 1975 with 17 species in 7 Genera ; Aromadendron 1, Kmeria 1, Magnolia 3, Manglietia 1, Michelia 6, Paramichelia 1 and Talauma 4. There are 14 native species and 3 introduced species. After the field and herbarium work on plant conservation project under the initiation of H.R.H. Princess Maha Chakri Sirindhorn since 1996 and reported in 2002 with 23 species in 3 Genera ; Magnolia 20, Manglietia 2 and Pachylarnax 1 due to the new name from proceedings of the international symposium on the family Magnoliaceae in Beijing in 2000. There are 19 native species and 4 introduced species. Five species of native Magnoliaceae are reported as new to the flora of Thailand. They are identified as *Magnolia compressa* Maxim, *Magnolia gustavii* King, *Magnolia mediocris* (Dandy) Figlar, *Manglietia insignis* (Wall.) Blume and *Manglietia utilis* Dandy. Where as *Magnolia gustavii* King and *Manglietia utilis* Dandy were reported as rare and endangered species.

P1459. The Phylogeny and Taxonomy of Magnoliaceae

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The family Magnoliaceae is one of the most primitive groups of angiosperms and has played an important role in the research of the origin, evolution and systematics of flower plants. In this paper author has reviewed the history and new progress of taxonomic and phylogenetic research, especially the controversial views and insufficiencies of current systems such as Y. H. Law's (1998) and R. B. Figlar & H. P. Nooteboom's (2004). Some important species with special taxonomic meanings are discussed and some new taxa are described. Moreover the new phylogenetic viewpoints, new cladogram and new system of the family are put forward. The system includes 3 subfamilies: Michelioideae (Law) D. L. Fu, subfam. comb. nov., *Magnolioideae* and *Liriodendroideae* (Bark.) Law, 5 tribes: *Michelieae* Law, *Yulanieae* D. L. Fu, trib. nov., *Alcimandrieae* (Law) D. L. Fu, trib. comb. nov., *Magnolieae* and *Kmerieae* D. L. Fu, trib. nov., 10 genera: *Michelia* L., *Paramichelia* Hu, *Yulania* Spach, *Alcimandra* Dandy, *Gynopodium* (Dandy) D. L. Fu, gen. comb. nov., *Magnolia* L., *Talauma* Juss., *Pachylarnax* Dandy, *Kmeria* (Pierre) Dandy and *Liriodendron* L..

P1460. The ancient flora fossil of México

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The ancient flora fossil of Mexico aged from the Superior Carboniferous, 320 million years, it is known as flora "Matzitz" and its location is in the state of Puebla on the central south part of Mexico. What characterizes this group is the Carboniferous worlds variety and rich typical flora to determine the age, it was based on the data of sub-overjacent rocks, on measures of stratigraphical sections, on geological mapping, on petrographical and paleobotanical studies. The flora, it is mainly made up of the: *Sigillaria* sp., *Sphenophyllum* sp., *Anularia* sp., *Calamites* sp., *Lepidodendron* sp., *Holcospermum* sp. genders, and of a significant group of *Marattiales*, as well as, of a similar branch of *Gingyophytas*. Currently the collection consists of more than one thousand specimen that were found within three hundred square kilometers. The similarity of its flora in regard to other latitudes (northeast of usa, southeast of Canada, France, Spain Germany, England, Poland, Czechoslovakia, and South Asia), permits to propose that the mexican flora is more related to the northern hemisphere (Laurasia) than to the southern hemisphere (Gondwana).

P1461. Taxonomical revision of Behuria Cham. (Melastomataceae), a endemic genus from Brazil.

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Its presents a taxonomic revision of the neotropical genus *Behuria*. The morphological study has been analysed the external morphology. Some anatomical characters of the leaves have also analysed to typify the trichomes and vascularization patterns. In the taxonomic treatment, 14 spp. are recognized, in which one taxon is described for the first time. It is provided a key to identify the species, morphological descriptions, illustrations, distribution maps, taxonomic comments, phenology and geographic distributional data of the taxa. For the first time, the occurrence of interpeculiar stipules is signed to *Behuria*, besides verticillate leaves. The center of genetic diversity of the genus is the Atlantic Forest vegetation in Rio de Janeiro state, where 10 spp. are found and 9 spp. are endemic. New synonyms are proposed: *Benevidesia* under *Behuria* and *Behuria mouraei* var. *rotundifolia* under *Bisglaziovia*. All species had their typus material characterized and some lectotypes were designated. Comments with close related genus, mainly *Bisglaziovia* and *Huberia*, are also presented.

P1462. Phylogenetic relationships of the Urticaceae, Cecropiaceae and Moraceae based of its comparative carpology

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Comparative morphological and anatomical study and cladistic analysis of the fruit diversity in the members of the most Urticaceae, Cecropiaceae and in several Moraceae genera is carried out. The results obtained by traditional methods support the distinction between three families studied. The "intermediate" family Cecropiaceae (Berg, 1978) is more closely related to Urticaceae, than Moraceae, which is in accordance with the opinion of Corner (1962) and recent molecular data (Systma et al, 2002, Datwyler & Weiblen, 2004). The Cecropiaceae is found to be heterogeneous taxon probably isolated early in the evolution of the Urticales. Contrarily, the cladistic analysis based on carpological data shows the derived position of the Cecropiaceae: the genera *Musanga*, *Cecropia*, *Coussapoa* belong to distinct Afro-American clade within the Urticaceae, whereas the position of the Australasian genus *Poikilospermum* remains unclear.

P1463. Cytoplasmic genotyping of *Musa* diploid and triploid accessions revealing hybrid origins and their geographical distribution.

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The *Musa* genome is constantly expanding with its high rate of somaclonal variations. The current classification based on morphological characters and predicted genomic constitution after crossing over makes this system unreliable for sterile,

heterozygous and polyploid *Musa* accessions. There is an urgent need of molecular markers system for screening of *Musa* accessions. Due to its uniparental inheritance cp and mtDNA provides an interesting and comprehensive tool for parental analysis of plants. In this study we have used cpDNA and mtDNA PCR-RFLP marker system revealing hybrid origins and geographic distribution of 48 *Musa* accessions using universal primers. So far we have detected 31 and 35 cytotypes with mtDNA and cpDNA markers respectively. Phylogenetic analysis provided the clear distinction between A and B cytoplasm and relationships between their polyploid progenies. The organellar genome analysis of *Musa* showed that the use of conserved gene markers for cp and mtDNA analysis is not only useful in determining inter and intraspecific variation but also for confirming hybrid origins and geographic distribution of *Musa* accessions.

P1464. Lineages in Banana Cultivars as Revealed by DNA Sequence Polymorphisms

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We have explored the use of polymorphisms at chloroplast (cp) and nuclear (nu) gene loci to determine the genealogy of cultivated bananas. Four cp marker loci; *rp16* and *ndhA* introns, *psaA-ycf3* and *petA-psbJ-psbL-psbF* intergenic spacers, and one nu marker corresponding to part of *Gbss* gene have been developed. *Musa acuminata* (A) and *M. balbisiana* (B)-genome derived alleles could be easily distinguished at each of the loci. Five maternal lineages were identified. Diversity within triploid hybrids was found and particular B-genome-rich triploids (ABBs) were given new genome designation as BBAs to indicate their maternal genealogy. Two *Gbss* loci were found in the investigation of the nu genome. Relationships of these bananas were inferred using neighbor joining and maximum parsimony methods. Our DNA sequence investigation opens the opportunities to easily trace lineages of contributing genomes within the banana cultivars, which subject has been puzzled crop scientists for decades.

P1465. New species in Myrtaceae and Leguminosae from Brazil.

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Two new species from Paraná State, Brazil are described: *Eugenia myrciariifolia* Soares-Silva & Sobral (Myrtaceae) and *Exostyles godoyensis* Soares-Silva & Mansano (Leguminosae). The first species is a small tree from seasonal forest of central and western Paraná, up 5 m high, with spatulate to linear cataphylls in base of younger twigs. The specific epithet is allusive to the resemblance of this species with some of the genus *Myrciaria*, especially *Myrciaria tenella* O. Berg, with which it can be easily confused when sterile due to its small leaves and dense foliage. The other one is the fourth species described in the small genus *Exostyles*. This species, from submontane semideciduous forest of the state of Paraná, is geographically disjunct from all the other species in the genus. It's a tree up 20 m high known only from type locality, required classification as critically endangered (CR) according to the IUCN (2001) categorization. The both species are illustrated.

P1466. Microsporogenesis and Gametogenesis in *Oncidium flexuosum* Sims and *Calanthe lyroglossa* Reichb. f. (Orchidaceae)

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Microsporogenesis and gametogenesis in two orchids, *Oncidium flexuosum* and *Calanthe lyroglossa* were studied with both Light and Electronic Microscopy, and the tissue processor, PELCO BioWave®, was chosen for fixation, dehydration, resin infiltration and staining the sample of *C. lyroglossa*.

The results show that the differentiation and development of pollen grains in pollinia are position related. In microsporogenesis, the tetrahedral and decussate tetrads make up the bulk of the pollinia, and the linear tetrads can only be found at the outside of the pollinia. In gametogenesis, the inner pollen grains with only intine

are major for pollen germination, and the outer pollen grains with tectum are major for protection. The pollen grains are differentiated into two types both in structure and function.

Meanwhile, we recommend to use microwave equipment that can keep pace with the ever-quickening need for rapid specimen turnaround while maintaining quality as high as ever.

P1467. Molecular phylogeny of *Phalaenopsis* Blume (Orchidaceae) based on the internal transcribed spacer of the nuclear ribosomal DNA

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The internal transcribed spacer of ribosomal DNA was sequenced from 53 species of genus *Phalaenopsis*. A phylogeny was developed for the genus based on the neighbor-joining and maximum parsimony analyses of molecular data. This result concurred that the genera *Doritis* and *Kingidium* should be treated as being parts of the genus *Phalaenopsis* suggested by Christenson (2001). Within the genus *Phalaenopsis*, both subgenera *Aphyllae* and *Parishianae* were not monophyletic and highly clustered with the subgenus *Proboscidioides* plus sections *Esmeralda* and *Deliciosae* of the subgenus *Phalaenopsis*. Those species also have the same characters of morphology of four pollinia and similar biogeography. Furthermore, both subgenera *Phalaenopsis* and *Polychilos* were also not monophyletic. Within the subgenus *Phalaenopsis*, only section *Phalaenopsis* was highly supported to be monophyletic. As to the subgenus *Polychilos*, only section *Polychilos* was moderately supported to be monophyletic. In conclusion, the present molecular data obtained from the ITS sequence of nrDNA of the genus *Phalaenopsis* provided valuable information for elucidating the phylogeny of this genus.

P1468. Detecting bias in host and fungal associates of three epiphytic *Aeridinae* orchids of Australia

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An epiphyte is an organism that utilizes another organism as a substrate upon which to live. Epiphytes derive no direct benefit from, nor do they confer any direct benefit to their host, thus most epiphytes are not host specific. Despite this, research on three epiphytic *Aeridinae* orchids of eastern Australia uncovered non-random distribution patterns within four disparate field sites. Two of the orchids, *Sarcochilus olivaceus* and *Plectorrhiza tridentata*, exhibited distinct host species preferences. All orchids depend upon a mycorrhizal fungus to germinate, thus it is intuitive that the host bias reflects a non-random fungal distribution. To test this hypothesis *Rhizoctonia*-like fungi were isolated from orchid roots from two field sites, pure cultures were obtained and the nuclear ribosomal ITS sequences of these isolates determined. The results suggest that these fungi are neither restricted to particular species of woody plants, nor to the different sites. They also demonstrate that these orchids associate with distinct clades of *Ceratobasidium* fungi and that their fungal associates correlate strongly with their ecological preferences.

P1469. Phylogenetic and geographical analyses of the orchid genus *Bulbophyllum* on Madagascar and the Mascarenes: can main speciation bursts be correlated with climatological, geological or ecological changes?

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The project proposed primarily aims a phylogenetic and biogeographic analysis of the Madagascan and Mascarenes species of *Bulbophyllum* (Orchidaceae), taking current and historical data into account.

The project proposes to apply three different techniques to determine whether main speciation bursts correlate with climatological, geological or ecological events. First, main clades

of closely related species will be identified and dated using phylogenetic analyses of DNA sequences and calibrations by vicariance. Secondly, analyses of distribution patterns using GIS will be performed to identify species-rich areas. Finally, possible correlations between main speciation events and climate, geology and ecological parameters will be investigated using phylogenetic-contrast analyses. The results will constitute an important evaluation of a major species-rich group of plants and provide a basis for designing areas deserving nature protection on Madagascar.

P1470. Intra- and inter-specific relationships in *Epidendrum* section *Amphiglottium* (Orchidaceae) inferred from AFLP markers

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The section *Amphiglottium* is an example of the variability that can be found in the genus *Epidendrum* L., with their polymorphic species, such as those in the *Epidendrum secundum* complex. The goal of this study was to clarify the relationships among the species of *Epidendrum* section *Amphiglottium*, and between them and the subsections *Carinata* and *Tuberculata*, by means of the AFLP (Amplified Fragment-Length Polymorphism) procedure. Three combinations of pairs of primers were employed (AC/CTA, TG/CTC, and AG/CTT) in 70 accessions. The data was processed by Cluster Analysis using UPGMA, with Jaccard's coefficient. The accessions of subsection *Carinata* were split into two groups: the first covers species from the Amazon Plateau, Andes, and the Brazilian Central Plateau, and the second contains species from the Atlantic seashore, and Southeastern Brazil. Subsection *Tuberculata* emerges as a different group, that were also split into two sub-groups: the first encompassing plants with lilac, orange, red or white flowers, that may be a single species - *E. secundum*; the second containing only plants with yellow flowers, which may also be a single species - *E. xanthinum*.

P1471. Circumscription and Interspecific Relationships of *Masdevallia* Subgenus *Pygmaea* (Orchidaceae - Epidendroideae - Pleurothallidinae)

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Pygmaea is the most taxonomically complex as well as confused subgenus in the genus *Masdevallia*. It includes 34 species grouped in four sections previously recognized as groups within the subgenera *Masdevallia* and *Amanda*. To examine the circumscription and resolve interspecific relationships of subgenus *Pygmaea* 20 taxa were investigated using DNA sequence data from the nuclear internal transcribed spacers (ITS) as well as morphological data. Parsimony analyses of both individual and combined data sets indicated that subgenus *Pygmaea* is polyphyletic. Its species are resolved as members of two well-supported clades: the first clade includes species of sections *Zahlbrucknerae* and *Amaluzae*, the second clade includes species of sections *Amaluzae*, *Aphanes* and a representative of section *Pygmaeae*, viz. *M. hojejeri*, recently transferred to a new genus, *Diodonopsis*. The second analyzed member of section *Pygmaeae*, *M. erinacea*, for which the genus *Diodonopsis* was erected, does indeed fall out of *Masdevallia* as previously published.

P1472. The "fly orchid" *Telipogon nervosus*: Morphology and anatomy of a bizarre regular flower in a bilateral neighborhood

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The Neotropical genus *Telipogon* (Orchidaceae-Epidendroideae-Maxillarieae) consists of ca. 100 spp., some of which have regular perianth. The genus was included (as a synthetic terminal taxon) by Freudenstein & Rasmussen (1999) but most floral characters remain missing or obscure. We studied the floral development, anatomy and morphology of *T. nervosus*, a high-Andean species, which exhibits regular perianth. The vasculature of the labellum is more complex than that of the lateral inner tepals, which are located below the labellum. Two different types of unicellular trichomes in the column are associated to the pseudocopulation syndrome of these orchids with Tachnid flies; the short trichomes are located mostly towards the base of the labellum, whereas the long ones are mostly around the single 2-celled anther. There are

4 pollinia attached to a tegula. The ontogeny of the inner tepals (associated to the regular symmetry), the anther, and the gynoeceum is discussed. Resupination is also discussed along with the inflorescence morphology. Finally, the structure of the ovary, including the massive, commissural structures along the carpels, is also discussed.

P1473. Rooting the Orchids: Testing root anatomy in the *Dryadella-Masdevallia-Porroglossum-Trisetella-Pleurothallis-Scaphosepalum-Platystele* clade (Orchidaceae: Pleurothallidinae)

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Colombia is one of the most diverse countries worldwide in the orchid family, with ca. 205 genera and 3 200 species. The subtribe Pleurothallidinae, with c 32 genera and 4000 species in the New World, is particularly rich in Colombia. Phylogenetic analyses in the Pleurothallidinae subtribe have been increased recently. Unfortunately the sampling is still limited in studies based upon both molecular (e.g. Pridgeon *et al.* 2001) and morphological (e.g. Neyland *et al.* 1995) data. Furthermore, these analyses have arrived at conflicting phylogenetic relationships of genera such as the New World *Porroglossum*. This genus is recognized as all their species have a remarkable tactile mechanism on their labellum. In a more inclusive level, Neyland *et al.* (1995) proposed some anatomical characters of the velamen as potential sinapomorphies of the clade formed by the genera *Dryadella* - *Masdevallia* - *Porroglossum* - *Trisetella* - *Pleurothallis* - *Scaphosepalum* - *Platystele*, all of them present in Colombia. In this work, we are testing in a phylogenetic context the anatomical characters related to the roots in a broader sampling of these genera, primarily from Colombian species.

P1474. The influence of habitat conditions on individuals characteristics of *Dactylorhiza fuchsii* (Druce) Soó in the Snieznik Massif (Poland)

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The research was carried in the Snieznik Massif (Sudeten Mts.) out in 1999-2005. The subject of the research was study of populations of *Dactylorhiza fuchsii*. This species grow in the meadows and pastures of class *Molinio-Arrhenatheretea*, water-logged spruce trees and broad-leaved forest of class *Quercus-Fagetetea*. This species characterizes great differentiation of individuals characteristics. This study plants divided in the 4 of groups. (I group) The plants that are growing in the alkaline and sunny habitat, have short and gnarly inflorescence (4-6 cm), leaves (6-8cm), often the colour of light green without spots. (II group) The plants are growing in the acid habitat, water-logged and shaded have long (8-12 cm) and narrow (1,7-2,5 cm) leaves the colour of dark-green for spots and loose inflorescence. (III group) This plants are growing in the alkaline and forest habitat, often water-logged have long and oval leaf, for dark spots. (IV group) This plants are growing acid and sunny habitat, length plants 30-45 cm. The study has been covered by the research study No.3P04 G 01224 in the years 2003-2006 funded by the Ministry of Research and Information Technology.

P1475. Taxonomical, Morphological, and Anatomical Investigations on Some Species of *Orchis* L. in Central Anatolia

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 In this study, the morphological, taxonomical and anatomical properties of three taxa of *Orchis* namely *Orchis mascula* (L.) L. subsp. *pinetorum* (Boiss. & Kotschy) G. Camus, *O. tridentata* Scop. and *O. coriophora* L., collected from center Anatolia, have been studied. It is determined that they mostly variable in size, shape of labellum, length of sepal hood, sepal shape and coloration of floral parts. The findings were compared with those in Flora of Turkey. In Turkey, for many centuries, various kinds of *Orchis* have been extracted from the tubers and exported under the name of *Sahlep*, hot drink during the winter. *Orchis mascula* and *O. coriophora* have the tubers entire and usually used for making the *Sahlep* and

ice-cream. They have widely distribution in Turkey but due to excessive gathering by indigenous people, their natural population have been decreasing in the course of time.

The studies on cross-sections of the root, stem, leaves and surface sections of leaves of these three *Orchis* members are also presented with their anatomical similarities and differences.

P1476. Contribution of Biomechanics to the analysis of anatomically-preserved fossil plants - a late Devonian example: the genus *Pietzschia* Gothan (Cladoxylopsida)

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The biomechanical approach developed by Speck and Rowe, aimed at determining growth forms characterizing terrestrial vascular plants, is applied to the study of the late Devonian anatomically-preserved genus *Pietzschia*(Cladoxylopsida). This genus includes three species on which we performed an architectural and developmental analysis. The latter resulted in plant reconstructions for two species, *P. schulleri* and *P. levis*. The third one, *P. polypsilon*, provided a number of structural informations. On this basis, the biomechanical properties of the three species were explored. Our work shows the essential role of Biomechanics in the study of anatomically-preserved fossil taxa, validating the preliminary reconstructions. A mixed biomechanical signal is identified in *P. levis*, originally reconstructed as an erect plant not exceeding 2 m high. A semi-self-supporting growth form is assigned to the large-sized species *P. schulleri*. Our analyses revealed that the typically-cladoxylopid dissected vasculature does not play a significant role in the stability of stems. The biomechanical approach also highlighted a number of ecological aspects.

P1477. *Condrusia* : a novel Famennian pre-ovulate structure.

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The genus *Condrusia* has briefly been described by Stockmans in 1948 as a putative seed. New specimens from six late Famennian (Upper Devonian) localities from Southern Belgium allow us to demonstrate that *Condrusia* possesses the traits that characterises pre-ovules.

The cupule of *Condrusia* is composed of two flat bilaterally symmetrical wings appressed against each other. The cupule is thought to have played a major role in the dispersion of the pre-ovule. Sections have allowed us to understand the structure of the pre-ovule body (integument, nucellus and megaspore) which is radiospermic. The sections further suggest that the integument is not dissected into lobes and show a complex organisation with three layers, the middle one consisting of large sclerified cells. The nucellus appears to be fused with the integument. Its apex is modified into a very long salpinx protruding above both the integument and the cupule.

Condrusia, with its bilaterally symmetrical cupule, its complete complex integument, and its very long salpinx, illustrates a novel and highly distinctive architectural type amongst the Upper Devonian pre-ovules.

P1478. Growth of the earliest land plants is promoted by epiphytic bacteria

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Liverworts, the most basal lineage of extant land plants, have been used as model systems in the reconstruction of adaptations to life on land. In this study we used gemmae (specialized propagules) that were isolated from mature gemma cups of two distantly related species of liverworts, *Marchantia polymorpha* L. and *Lunularia cruciata* L. (order Marchantiales). We show that methylobacteria (genus *Methylobacterium*), microbes that inhabit the surfaces of land plants where they secrete certain phytohormones, promote the growth of isolated gemmae cultivated on agar plates. As a control, two species of higher plants, maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) were raised aseptically from sterile seeds (i. e., caryopses, achenes). Inoculation of these propagules with methylobacteria was without effect on growth in the above-ground phytosphere (expansion of stems and leaves). We conclude that normal development of the "living fossil" - plants *Marchantia* and *Lunularia* is dependent on

(and possibly regulated by) epiphytic methylobacteria, whereas higher plants grow at optimal rates in the absence of these prokaryotic epiphytes.

P1479. Adaptive Strategies of Aquatic Basal Angiosperms and Monocots

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We explore the adaptive strategies of representative families of aquatic plants from the Basal Angiosperms through Monocots in order to explain the basis for the prevalence of aquatic Monocots, especially rooted floating and emergent macrophytes, and their dominance of certain wetlands. In the existing habitats of these plants, clonal reproduction is common whereas seed production leading to seedling establishment occurs more often in new habitats. The plants dominate their habitats by clonal reproduction and appear to use seed reproduction most successfully in extending their habitats. Successful adaptive strategies involve overcoming anoxic conditions imposed upon their roots, transport of oxygen from aerial environments to anoxic root conditions, and evolution of root/shoot aerenchymatous systems. The occurrence of cortex modifications of aerenchyma and barrier layers, endodermis and hypodermis (exodermis), is characteristic not only of roots, but also of shoots in the substrate or water for many plants. It also appears that many exhibit phenotypic plasticity, especially in some of their anatomical and morphological characters.

P1480. Application of petiole anatomy in taxonomic problems and relationships of some *Peucedaneae* and *Smyrnieae* (Apiaceae) in Iran

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Opopanax hispidus and *Smyrniopsis aucheri* (*Smyrnieae*) have been placed in two different tribes (sensu Drude), but the recent classification based on cpDNA sequences has shown that they constitute a monophyletic group. This study also showed that they shared in some significant character.

Opopanax with *Laser* and *Leutea* with *Dorema* based on serotaxonomical researches considered as a close genera. This study also showed that they have a similar characters.

The monotypic genus *Azilia eryngioides* has also some common character with *Leutea*.

Another monotypic genus *Hausknechtia elymaitica* that was supposed synonym or very close to *Dorema*, this study clearly showed that they are very far from each other.

Members of *Peucedanum* complex based on recently cpDNA sequences constitute a polyphyletic group, but all members in Iran have a common character. However, in comparison with other species of the other part they don't show a similarity.

P1481. Study of effects plant density on quality characterizes in wheat (*Triticum aestivum*.L)

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In order to evaluating of row spacing and seed value on quality characterizes in Kohdasht wheat (*Triticum aestivum*.L.) and study about new cultivar an experiments was conducted under dry land farming in experimental farm of Agricultural Research Center of Gonbad (Golestan province- Iran)in 2003-2004(farming year).The experiments was arranged as randomized complete blocks design (statistical methode)with three replication (1200 m²).The treatments resulted from the factorial combination of three row spacing (12.5 , 17and 25 cm) and four seed value (250, 300, 350 and 400 seed per m²). After experiment , in chemical labrotary for plants and with SAS program ,Zeleny index was significantly affected by different seed value .And water adsorption percentage was significantly by interaction effect. Study of results showed the best row spacing is 17 cm and seed value is 300-350 seed per m²

P1482. Winter wheat yield increase through wheat, alfalfa, fallow, soybean rotation and application of manure in Varamin province in Iran

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A two year (2001-2002) experiment was conducted to study the effect of winter wheat rotation with legume crops and the effect of fallow and different amounts of manure on success of winter wheat crop in the region of Varamin. The experiment was initiated on a land laid fallow for the two precedent years, and was arranged in a split plot design with winter wheat, annual alfalfa, soybean and fallow as main plot and manure levels (0 and 5 t/ha) as subplots. In 2001 a single wheat crop was cultivated and a two factorial experiment in four replications was carried out. The manure and rotation effects were positively significant on winter wheat yield and its components. In rotation of wheat, fallow, soybean, alfalfa, 1860, 2579, 4080 and 4830 kg/ha of grain yield in manure treated plots and 1490, 2090, 3130 and 4100 kg/ha of grain yield in no manure plots were obtained respectively. The results of this study showed that using winter wheat yield in rotation with annual alfalfa and soybean with manure application brought considerable increase in yield.

P1483. Geographical pattern in genome size of *Festuca pallens*: phylogeographical interpretation of distribution range of polyploid complex.

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Considerable genome size variability in both ploidy levels and at various spatial scales was detected in *Festuca pallens* Host using DAPI stained flow-cytometry. Within- and among related sites, DNA content was relatively homogenous. However, mean differences up to 5.9 % between nearby localities or up to 11.6 % within the populations were rarely detected. Within the whole distribution area, maximum differences 15.9 % (in diploids) and 14.0 % (in tetraploids) were observed. In both ploidy levels, genome size was correlated with geographical coordinates, with the largest genomes situated in the southeastern parts. Mutual geographic relation between DNA content of diploids and tetraploids was confirmed. Tetraploids have in general lower DNA content than expected by doubling of their diploid progenitors. The main differences in DNA content of tetraploids were attributed with affiliation to 3 different, taxonomically unevaluated types. Affinity of larger genomes to different types of glacial vegetation (steppes rather than periglacial tundra, together with relation to relict habitats in diploids, give reasons for phylogeographic interpretations.

P1484. C3 photosynthesis in *Aristida longifolia*: Implication for diversification of photosynthesis in Aristidoideae (Gramineae)

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Aristida (ca. 300 species) is one of three genera in Aristidoideae (Gramineae) along with *Sartidia* (4) and *Stipagrostis* (50). The genus is distributed in the tropics and subtropics, growing on grassy plains, arid lands, and open ground. Studies of this taxonomically difficult genus have relied primarily on morphology and anatomy. *Aristida* and *Stipagrostis* carry out C4 photosynthesis (NADP-ME and NAD-ME, respectively), whereas *Sartidia* undergoes C3 photosynthesis. A survey of the leaf blade anatomy of Aristidoideae and carbon isotope ratio tests has revealed the presence of non-Kranz anatomy and C3 photosynthesis in *Aristida longifolia* Trin. This species is distributed from Central to South America and grows in partial shade along margins of gallery forests. This discovery represents the second report of non-Kranz anatomy and C3 photosynthesis in Aristidoideae. The first report involves species of *Aristida* now treated in the segregate genus *Sartidia*. Molecular phylogenetics of Aristidoideae indicate that *A. longifolia* does not form a clade with *Sartidia* but is sister to the remaining *Aristida*, and that C3 photosynthesis is likely plesiomorphic.

P1485. Phylogenetic relations of the South American species of *Rytidosperma* Steud. (Danthonioideae, Poaceae) based on morphoanatomical characters

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The phylogenetic relations among 36 species of *Rytidosperma* Steud. (Poaceae) were inferred from 21 morphological and anatomical characters. The matrix was elaborated from direct measurements and observations of the six South American species *R. lechleri* Steud., *R. pictum* (Nees et Meyen) Nicora, *R. quirihiuense* C.Baeza, *R. sorianoi* Nicora, *R. violaceum* (Desv.) Nicora, *R. virescens* (Desv.) Nicora besides Easter Island's *R. paschale* (Pilg.) C. Baeza. They were after put together in the matrix with the rest of the *Rytidosperma* species used by Linder and Verboom (1996). Parsimony Analysis were applied through the Ratchet Method utilising Winclada ver. 1.00.08. *Danthonia unispicata*, *Danthonia malacantha* (Poaceae), *Tribolium pusilla* (Poaceae) and *Schismus inermis* (Poaceae) were considered as outgroups, rooting the cladogram from the former species. The results support the hypothesis that the South American species conform a monophyletic clade.
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P1486. Revision of *Monnina* (Polygalaceae)

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Monnina (Polygalaceae) is a genus of herbs and shrubs distributed from southwestern United States to southern South America. It occurs from sea level to 4100 m in both open and forested areas. It has three subgenera, about 150 species and almost 400 names. Although there are floristic treatments for species from North and Central America, Brazil, Colombia, Ecuador, Peru, Venezuela, and a century old taxonomic treatment, there is no comprehensive monograph of *Monnina*.

At least for identification purposes, these morphological characters seem to be the most informative: extra-floral nectaries, habit, stems and leaves vestiture, external sepals, fruits, and filaments. In addition, most of the species with similar flower morphology seem to have two character suits: 1) herbaceous plants, lax inflorescences, dry fruits and lowland distribution; and 2) woody plants, dense inflorescences, fleshy fruits and highlands distribution. A third group of species also has herbaceous plants with lax inflorescences and lowland distribution, but it has fleshy or dry fruits and different flower morphology.

P1487. Molecular phylogeny of the Polygonaceae based on chloroplast *matK* Sequences

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Polygonaceae comprises approximately 1000 species in about 40 genera. Most species within the family show very complicated patterns of morphological variation, and many different infrafamilial classification schemes have been proposed. We have analyzed chloroplast *matK* sequences from 75 species representing 30 genera using maximum parsimony to infer phylogenetic relationships among the taxa within the family. The strict consensus tree obtained strongly suggests that Polygonaceae and subfam. Eriogonoideae are monophyletic. However, subfam. Polygonoideae appears to be paraphyletic. Of the five tribes recognized within subfam. Polygonoideae, only Persicarieae, Rumiceae, and Triplareae are monophyletic; *Muehlenbeckia* and *Fallopia*, traditionally placed in tribe Coccolobeae, are nested within Polygonaceae. At the generic level, *Polygonum* s. str., *Fallopia*, and *Chorizanthe* appear to be nonmonophyletic.

P1488. The evolution of reproductive systems and sex-determining mechanisms within *Rumex* (Polygonaceae) inferred from nuclear and chloroplastial sequence data

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We compare nuclear ITS and chloroplast intergenic sequences of 31 species of *Rumex*. Our phylogenetic analysis supports a systematic classification of the genus, which differs from that currently accepted. This new phylogeny suggests a common origin for all Eurasian and American dioecious species of *Rumex*, with gynodioecy as an intermediate state on the way to dioecy. Our results support the contention that sex determination based on the balance between the number of X chromosomes and the number of autosomes (X/A balance) has evolved secondarily from male-determining Y mechanisms and that multiple sex chromosome

systems, XX/XY₁Y₂, were derived twice from an XX/XY system. The resulting phylogeny is consistent with a classification of *Rumex* species according to their basic chromosome number, implying that the evolution of *Rumex* species might have followed a process of chromosomal reduction from $x=10$ toward $x=7$ through intermediate stages ($x=9$ and $x=8$).

P1489. The shoot architecture in Portulacaceae

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The Portulacaceae includes about 20 genera and more 500 species. The structure of inflorescences and shoot has not been investigated good yet. We report here the data of shoot system organisation of *Talinum paniculatum*, *T. triangulare*, *Calandrinia grandiflora*, *C. compressa*, *Portulaca oleracea*, *P. cryptopetala*, *Claytonia sibirica*, *C. acutifolia*, *Montia fontana*. The shoot architecture of some Portulacaceae does not unequivocally fit the Troll's model of the inflorescence. Then, modules of the shoot systems have been recognised which are different in various representatives. In all Portulacaceae except for *Claytonia*, module are terminated by the flower and sympodially disposed. *Claytonia* strikingly differs in monopodial rosette shoot bearing axial monochasia. *Claytonia* is a separate genera from *Montia*. The two genera might be placed into separate subtribes or tribes. The project is carried out under financial support of RFBR, grant N 04-04-49010.

P1490. Preonanthus: An Independent Genus Formed by Alpine Ranunculaceae Taxa

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Pulsatilla, genus of Ranunculaceae family, contains more than 170 taxa, but the objective correlations between these categories are still unclear. The morphologically variable species make the correct classification really difficult. However, there are some taxa (*P. alpina* and its subspecies, variants), which show significant morphological difference from other *Pulsatilla* species. We tried to support this difference with phylogenetic studies. Molecular phylogeny was constructed using nucleotide sequences of internal transcribed spacer (ITS) regions of nr DNA. A phylogenetic tree was constructed from the nucleotide sequence alignment using PAUP* 4.0. ITS regions seem to be valuable source in identifying different species, while significant polymorphism manifested in most of the studied taxa at species level. 2 samples showed significant polymorphism at generic level. While these species are exactly the same, which differ in numerous morphological characters from all the other *Pulsatilla* taxa and were classified as *Preonanthus* species, we think these taxa should be considered generically different and *Preonanthus* genus should be recalled.

P1491. Phylogeny and evolution of Ranunculus s.l. (Ranunculaceae)

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Ranunculus belongs to the 40 largest plant genera of angiosperms, comprising c. 600 species (plus c. 600 agamospecies) and occurring worldwide in a broad range of habitats. Phylogenetic reconstruction using DNA sequences (nrITS, *matK-trnL*) is incongruent with previous classifications due to homoplasy of morphological characters. Combined molecular data suggest a generic delimitation excluding *Ficaria*, *Ceratocephala*, *Myosurus*, *Beckwithia*, *Halerpestes*, *Peltocalathos*, *Callianthemoides*, and *Arcteranthis*. On infrageneric level, clades correspond to widespread ecological or regional geographical groups, and to basic chromosome numbers. Age calibrations suggest an age of *Ranunculus* s.s. of c. 24 Myr, a diversification into main ecological/geographical clades in the late Miocene and into present species during the Pliocene and Pleistocene. Split decomposition analyses suggest reticulate evolution within several groups. The evolutionary success of the genus is probably caused by adaptation due to high morphological plasticity and a broad

range of reproductive strategies, plus hybridization and polyploidy as important factors for speciation.

P1492. Rose: a model system to study abnormal somatic embryogenesis

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Study on developmental and structural aspects of somatic embryogenesis has received great attention in recent years. Information on developmental regulation of somatic embryogenesis at molecular level has started to flow. However, little progress has been achieved toward understanding the switching over cell programming from normal process of somatic embryo development to abnormal somatic embryogenesis. Moreover, in certain cases abnormality were induced to study the alteration in cell programming. In the present investigation, *Rose*, a woody ornamental plant was taken up to study somatic embryogenesis. The zygotic embryos harvested at different developmental stages were subjected to various concentrations of 2,4-D and physical conditions. During this process a wide range of abnormal embryos were observed. The origin of this abnormal somatic embryogenesis was elucidated by electron microscopy. The stage of shift of their developmental pattern was also identified by comparing it to the normal somatic embryo development in angiosperm. In the present study, the diverse kinds of abnormal embryos as well as the ease in obtaining them strongly advocates the use of **Rose plant as a model system** to study abnormal somatic embryogenesis and to address various unanswered questions on plant morphogenesis.

P1493. The endocarp of European Rubus-species

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In Europe 4 subgenera of the genus *Rubus* (subgen. *Chamaerubus*, *Cylactis*, *Idaeobatus* and *Rubus*) and several hundred species occur.

Usually morphological features are used to characterize the taxa. Not taken into further consideration is the endocarp (seeds). It consists of two layers strongly sclerenchymatous cells, one right-angled, the other parallel to the longitudinal axis. Diagnostic relevant features are surface (mostly reticulate), shape and size. The herbaceous species of the subgen. *Chamaerubus* and *Cylactis* are characterised by a smooth surface (*Rubus chamaemorus*, *R. arcticus*) and (except *R. arcticus*) big seeds. Similar to in size and shape, but significant smaller is *Rubus caesius* (subgen. *Rubus*, sect. *Caesii*). The reticulum is less prominent than in *Rubus* subgen. *Idaeobatus* and *Rubus* sections *Rubus* and *Corylifolii*.

Rubus idaeus (Subgen. *Idaeobatus*) shows rather small to medium sized seeds, whereas the endocarp of the brambles (subgen. *Rubus*, sect. *Rubus*) is much more variable. A further differentiation of species of the sections *Rubus* and *Corylifolii* is possible when all features are taken into consideration.

P1494. Evolution of Nertera (Rubiaceae) with focus on the Tristan da Cunha archipelago

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The genus *Nertera* is distributed from SE-Asia to South America and the isolated Tristan da Cunha archipelago. ITS and trnL-F sequences and AFLPs were analysed to answer questions from species to population level. Results suggest that the genus originated in New Zealand and subsequently spread into the Pacific area. *Nertera depressa*, the only widespread species, is genetically diverse and can be divided into three geographical groups: (1) Southeastern Asian Group, (2) Northern South American Group, (3) Southern South American Group. From the latter it dispersed to Tristan da Cunha were the two endemic species *N. assurgens* and *N. holmboei* evolved. Morphology and taxonomy were found to be incongruent with the genetic relationships. *Nertera depressa* from Tristan da Cunha is genetically more closely related to the two endemic species than to *N. depressa* from Chile and the Falkland Islands, and within the archipelago *N. depressa* from Inaccessible is genetically closer to the endemics than to *N. depressa* from Gough and Tristan. Both

endemic species seemingly evolved on Inaccessible when Tristan, the largest island in the group, was not yet in existence.

P1495. The chromosome study of *Argostemma* spp. (Rubiaceae) from Thailand.

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Abstract: The new information on the chromosomes of selected species in the genus *Argostemma* Wall. (Rubiaceae) from Thailand had been achieved. Concerning the fact that there are two major groups of *Argostemma* due to the morphological characters i.e. the group with bell-shaped flowers and the other with star-shaped flowers. The former had centre of origin in peninsular Malasia while the later had distribution ranges mostly in Thailand and neighbouring areas. It is to be doubted whether the genus should be separated into two subgenera due to this distinct characteristics or not. Chromosomes of selected species of *Argostemma* from Thailand, mostly from the peninsula, had been investigated. Most species have the same number as $2n=22$. The relationships between species in the genus due to the chromosome number and karyological characters had been discussed. It is suggested here that *Argostemma* Wall. might remain a "good genus". However, more information on chromosome numbers together with the karyotype patterns of some selected species would be needed in order to support the relationships between various taxa in the genus.

P1496. Is the Origin of *Veronica* L. Genus Turkey?

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In this article, we tried to explain the distribution of *Veronica* genus throughout the world that distributed from Turkey. This subject has been considered in this article firstly as original and scientific. Over 80 out of 200 species of *Veronica* genus are found in Turkey which is small region comparing to world land and endemism rate is very high. This suggest that *Veronica* species on the World may originate from Turkey. The richness of this species may be affected by different factors those are different kind of geomorphologic structure and climate and microclimate in the region, specific sediments belong to geologic period and positioned in the moderate zone as a geographic situation and even paleomorphologic, paleogeographic and paleoclimatologic changes. Also, historical, ethnographical and some other natural factors have contribution to this distribution. Since a small region of Turkey comparing world area contains high number of *Veronica* taxa. Turkey is situated in the geometrical center of the distribution of *Veronica* in the earth. It can be concluded that Turkey is the original centre of *Veronica* Genus, gene pool according to scientific and theoretic data.

P1497. Notes on the genus *Orobanche* in Italy, 1. Taxa described by Bertoloni

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In order to prepare the revision of the genus *Orobanche* in Italy, the 11 species as new described by Antonio Bertoloni, in 1810 and 1846, are considered. Five of them were described on material collected by Bertoloni himself; the other, on specimens received from Vaucher, Moris and the younger Savi. Subsequently, all names proposed by Bertoloni were relegated to synonymy. The revision of the original material housed in BOLO results as follows: *O. bicolor* Bertol. (non Meyer) belongs to *O. cernua* L., *O. cruenta* Bertol. to *O. gracilis* Sm., *O. stricta* Moris ex Bertol. to *O. schultzei* Mutel, *O. thyrsoides* Moris ex Bertol. to *O. rapum-genista* subsp. *rigens* (Loisel) P. Fourn., *O. critmi* Bertol. to *O. minor* Sm., *O. vitalbae* Bertol. to *O. picridis* F. W. Schultz, *O. fragrantissima* Bertol. to *O. lutea* Baumg., *O. laurina* Bertol. to *O. hederæ* Duby, *O. yuccae* Savi f. ex Bertol. to *O. hederæ* Duby [not to *O. minor* Sm. as had been suggested], *O. centaurina* Bertol. to *O. litorea* Guss. [and not to *O. picridis* F. W. Schultz]. However, *O. australis* Moris ex Bertol., referred by Beck to *O. canescens* C. Presl, appears to be a good species so far known only from Sardinia.

P1498. Variation trends in the genus *Melampyrum* (ex-Scrophulariaceae)

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The genus *Melampyrum* belongs to the most variable plant genera in Europe. The indistinct species limits in some groups are a source of taxonomic uncertainties at the interspecific level. Most conspicuous at the intraspecific level is so-called "seasonal variation", which is traditionally evaluated as the most important taxonomic character at this level. The ecological plasticity and relationships with host-plants are another source of the intraspecific variation.

A detailed morphometric study of model species belonging to groups with different patterns of inter- and intraspecific variation was performed. Some morphologically intermediate types in groups of *M. nemorosum* and *M. sylvaticum* indicated a possibility of hybridisation, which may result in morphologically intermediate types in some groups. This hypothesis was supported by hybridisation experiments.

Analyses of intraspecific variation in *M. nemorosum* and *M. pratense* show different patterns of seasonal variation in these species. The structure of seasonal variation tends to have reticulate pattern in some species but constitutes distinct clusters of similar types in other species.

P1499. Eco-geographic distribution of ploidy levels in wild and cultivated potatoes (*Solanum* section *Petota*)

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Wild potatoes (*Solanum* sect. *Petota*; 187 species) occur from the SW USA to Chile. There are diploids, triploids, tetraploids, pentaploids and hexaploids; cultivated potato (*Solanum tuberosum*) has all these levels except hexaploid. We compiled 8697 ploidy reports, including 912 new determinations, and analyzed the frequency and the eco-geographic distribution of species with different ploidy levels and of cytotypes within a species. Thirty-six percent of the wild species are entirely or partly polyploid. Multiple cytotypes exist in 22 species. There are more diploid species, and they occupy a larger area than the polyploids but tetraploid species have, on average, similar range sizes as diploid species. Polyploid species frequency is much higher from Mexico to Ecuador than further south. Compared to diploids, triploids tend to occur in warmer and drier areas, while higher level polyploids tend to occur in colder areas. There are no diploids from Costa Rica to Colombia, the wettest part of the group's range. Tetraploid cytotypes of *S. tuberosum* occur much more frequently than the diploids; the triploids and pentaploids are restricted to colder and drier areas.

P1500. Synopsis of *Solanum* subgenus *Leptostemonum* in Brazil

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Solanum subgenus *Leptostemonum* is the second largest of the *Solanum*, with approximately 450 species and cosmopolitan distribution having its primary center of diversity in South America and secondary centers in Australia and Africa. Morphological characters used to distinguish it from other subgenera include: stellate indument, prickly herbage, and attenuate anthers. In Brazil approximately 251 specific names have been applied to the species, of which, 131 are recognized as synonyms. *Solanum* subg. *Leptostemonum* is represented in Brazil by about 120 species, of these, 45 are endemic, including the undescribed taxa. They are assigned to the following sections: *Acanthophora*, *Crinium*, *Melongena*, *Erythrotrichum*, *Herposolanum*, *Lasiocarpa*, *Micracantha*, *Persicariae*, *Polytrichum* and *Torva*. They are encountered in many kinds of vegetation and display marked adaptations to humid, dry, hot and seasonal habitats. The principal center of diversity and endemism in Brazil is found in South-

eastern coastal region, which has 67 species, representing 61% of the total, of which, twenty two are endemic to the area.

P1501. Anatomy of foliar epidermis in species of Solanum Section Geminata (Solanaceae)

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Solanum falconense, *S. tanysepalum* and *S. lucens* of the *S. arboreum* group; *S. imberbe* and *S. sieberi* of *S. deflexiflorum* group were studied in the context of an ongoing anatomical research in the Geminata section. Material was treated using conventional anatomical procedures. Location, type and distribution of the stomata and trichomas, shape and wall thickness of the normal epidermal cells were evaluated. The species were amphistomatic with anisocytic and staurocytic stomata, being the late less frequent. Trichomes were located on the abaxial and adaxial surface. Glandular trichomes were present in *S. imberbe*, *S. sieberi*, *S. lucens* and *S. tanysepalum*. Only *S. falconense* had mixed trichomes in both surfaces. Epidermal cells differed depending of their location on adaxial or abaxial surface, being sinuous in the abaxial surface in all studied species. The species *S. falconense*, *S. sieberi* and *S. imberbe* exhibited polygonal anticlinal cell walls on the adaxial surface, while *S. lucens* and *S. tanysepalum* presented undulate anticlinal cells walls. This results may represents a contribution to the understanding of species in the section Geminata.

P1502. Verbenaceae from Guerrero, Mexico, morphology and pollen.

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The taxonomic knowledge above the Verbenaceae family in Mexico it's so far to be complete, actually there is just a fragmented and incomplete record of this vascular plant group. There are a few known studies about the taxa delimitation and their relationships, and those are supported on morphological characters judgments for taxa delimitation. In this project 15 genera and 48 species in Guerrero state distributed were studied and mean all of them interesting taxonomic cases and approaches for the Mexican flora knowledge, as the *Lantana* genus and its so many specific and infraespecific taxa. For this reason we decided to employ the palinological morphology as the aim to carry on findings more analysis elements in addition to morphology. The obtained results show that the genera studied does present particular morphological characteristics and some of them can be supported with the palinological morphology, however the great number of the specific taxa doesn't have sharp and precise palinological characters.

P1503. La familia Verbenaceae en Guerrero, México: morfología y polen.

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The taxonomic knowledge about the Verbenaceae family in Mexico it's so far to be complete, actually there is just a fragmented and incomplete record of this vascular plant group. There are a few known studies about the taxa delimitation and their relationships, and those are supported on morphological characters judgments for taxa delimitation. In this project 15 genera and 48 species in Guerrero state distributed were studied and mean all of them interesting taxonomic cases and approaches for the Mexican flora knowledge, as the *Lantana* genus and its so many specific and infraespecific taxa. For this reason we decided to employ the palinological morphology as the aim to carry on findings more analysis elements in addition to morphology. The obtained results show that the genera studied does present particular morphological characteristics and some of them can be supported with the palinological morphology, however the great number of the specific taxa doesn't have sharp and precise palinological characters.

P1504. New insights in high altitude environments evolution inferred through a phylogenetic hypothesis of *Lippia* spp. (Verbenaceae).

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We built a phylogenetic hypothesis for 12 species of *Lippia* which occur in the Espinhaço Chain of Minas Gerais, a high endemism area. In our tree, most of the basal branches were very short while the terminals were long, indicating that most variation is not shared by different species. It can be explained by the lack of phylogenetic signal, which could be related with rapid speciation events after the Last Glacial Maximum. It is also possible that the species present in high altitudes are just descendents of different species that reached these environments and became adapted to them. Another possibility is the lack of resolution of the ITS and Trn-L sequences. Regardless the lack of resolution of the tree, we found a lack of phylogenetic agreement of the sequences and the number of chromosomes. On the other hand, our tree did not disagree with the morphological classification within the genus, which indicates that morphological characters retain phylogenetic signal in addition to the expected convergence.

P1505. Species delimitation within the *Zamia verschaffeltii* Miq. species complex (Zamiaceae-Cycadales)

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The cycad *Zamia verschaffeltii* Miq. (1870) was described from material collected in Mexico by Ferdinand Deppe and Christian Wilhelm Schiede, German plant collectors who explored and collected in Mexico and Central America between the years 1828 and 1829. However, owing to unprecisely recorded locality information on the holotype of *Z. verschaffeltii* the type locality still remains an enigma (Socorro), but it is suspected that it is within south eastern Mexico in the states of Veracruz, Tabasco or Chiapas where the following species of *Zamia* are known: *Z. loddigessii*, *Z. cremnophila*, *Z. purpurea*, *Z. variegata*, *Z. lacandona*, and *Z. splendens* the latter two species are thought to be in synonymy under *Z. verschaffeltii* = *Z. katzneriana*. Two localities under the name "Socorro" have been detected, one in Tabasco and another in southern Veracruz. Preliminary results from morphometric and phylogenetic analysis indicates that *Z. splendens* is a different taxonomic entity to the *Z. verschaffeltii* concept observed by Miquel

P1506. Current research on the Mexican cycad flora

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Mexico is second world wide in cycad diversity with 45 species described to date among three genera: *Ceratozamia*, *Dioon* and *Zamia*. Advances on preliminary molecular phylogenies in the genera *Ceratozamia* and *Dioon* has indicated that good resolution for species delimitation is still problematic, but biogeographic inferences have been made. Multivariate analysis of morphological data is helping to clear up some taxonomic confusion in a *Zamia* species group in south eastern Mexico. Population genetics of two *Dioon* spp distributed along the Sierra Madre Oriental have revealed two distinct distribution patterns associated with genetic bottleneck effects and Pleistocene floristic refugia. Though chromosome number and karyotype is known to be highly variable in *Zamia* and conservative in *Ceratozamia* there appears to be a correlation in number of chromosome satellites with latitude in *Ceratozamia*. These topics are discussed in biogeographic and evolutionary terms.

P1507. Type specimens of Croatian endemic *Limonium* species (Plumbaginaceae)

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In Croatian flora the genus *Limonium* Mill. (Plumbaginaceae) is represented by 11 taxa. On the basis of literature, field and

herbaria studies original type specimens has been traced in different herbaria (WU, W, ZA, BOLO and PRC). The historical review of taxa treatments and the typification process are briefly discussed for *Limonium cancellatum* (Bernh. ex Bertol.) O. Kuntze, *L. dictyophorum* (Tausch) Degen, *L. subanfractum* Trinajstic, *L. vestitum* (C. E. Salmon) C. E. Salmon and *L. vestitum* subsp. *brusnicense* Trinajstic. The specimen from the WU herbaria was selected as the lectotype of *L. vestitum*. Original holotype material of *L. cancellatum* is deposited in BOLO herbarium and of *L. subanfractum*, *L. vestitum* subsp. *brusnicense* in ZA herbarium. We found and discussed existence of two type specimens of the same species, firstly described as *L. dictyophorum* in PRC herbarium and than as *L. anfractum* in WU herbarium. According to the ICBN, specimen of *L. dictyophorum* was chosen as adequate type material.

P1508. Effects of farm manure, inorganic nitrogen and Rhizobium on yield of soybean (*Glycine max* L.)

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An experiment was carried out for two years (2001 - 2002) to determine the effect of organic manure, inorganic nitrogen and *Rhizobium* bacteria on soybean 'Williams' yield, under semi - arid condition in the region of Varamin. The effect of organic manure in 3 levels inorganic nitrogen in 3 levels, and *Rhizobium* strains consisting of 3 commercial forms was investigated using a split plot design with 4 replications.

The results of yield analysis and other characteristics of soybean showed significant differences between factors and between the two years of experimentation. The results showed that soybean seed inoculation with bacteria strains has increased the crop yield and differences among strains were observed in different conditions.

Application of different levels of organic nitrogen led to different amount of yields in presence of nitrogen fertilizer levels, normal distribution of rain and timely sowing of crop in 2002.

The results of this experiment showed that application of organic nitrogen and inoculation of soybean seed with appropriate bacteria will lead to yield increase of this crop.

P1509. Regressive-informational model of plant diversity origin

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The model explains modern plant diversity on Earth. It is based on four statements. First, all plant diversity originates from several dozens of plant species (prototypes). In the whole history of Earth, plants accommodated to changes in atmosphere, temperature and soil chemical composition. They were transformed according to a set of multivariate information. Second, in genotype of modern plants there is an information not only about synthesis molecular components of cell, but also about mechanisms that assemble them to complex and organs, response reactions to environment and cosmos. Third, there are no meaningless nucleotide sequences in DNA. The information is coded not only by the nucleotide triplet but also by binary codes on DNA surface. Information transforms not only from DNA to proteins, but from different signals to DNA. Fourth, new plant species and subspecies were formed during the settlement on Earth by adapting to the environment conditions and simplifying genome prototypes' (sometimes complicating genome prototype). It led to habitus, morphology, physiology and biochemistry changes. The botanists register this as a new species appearance.

P1510. Plant Biodiversity of Nara Desert of Sindh, Pakistan, in terms of Microhabitat

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One hundred sixty plant species, belonging to 118 genera and 45 families were collected from the Nara desert during 1998-2001 are identified. Of them, one species of gymnosperm, three sedges and twenty species of Poaceae (grasses) are determined. The checklist is arranged alphabetically along their vernacular names, family and habitat.

Statistically the area containing the 21.22 %, of the plant families, 7.81% of the genera and 2.94% of the total species of Pakistan has been so far revised by Ali et al. (1971-2002).

Six distinct habitats were identified for plant communities from the study area: Crest habitat (Top of the sand dune); Slopes/swale/flanks habitat; Sandy plains Habitat (*Tar-Tarai*- low laying flat areas); Lake/wetland habitat; Saline/Sodic Land and Hilly Tract.

Floristically the crest habitat possesses 14 species (8.75%) a less number of species as compared to the rest of habitat. The highest number of species 103 (66.25%) recorded from the Flat Habitat.

P1511. The threatened plants, lost habitats and endemic species in the flora of Sanliurfa (Turkey)

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The natural living areas (habitat) in our country, as if in the World, have been constantly destroying and decreasing, because of the various antropogenic activities. This proses, have been limiting and the natural plants species, as weel as destroying the genetical sources must be prevented or become slow immediately. However, GAP Region has not been researched widely and the habitats of this region has been constantly changed. In the natural flora of the region, rare and endemic plant species have been concerned and factors which threated these species and danger categories have been observed. In this study, we investigated the feature of natural flora of GAP region. Recently, there has been increased harmful antropologic factors thretnening the future of and the destroying the natural flora of the region. Therefore, the endemic species in the region have been under great danger of extinction.

P1512. The Morphology, Type, Frequency and Distribution of Calcium Crystals in Some Medicinal Plants of the Philippines

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The morphology, type, frequency and distribution of calcium crystals in some Philippine medicinal plants was determined using light microscopy. Representative plants from the Amaranthaceae, Solanaceae, Fabaceae, Cucurbitaceae, Umbelliferae, Cruciferae, Convulvulaceae and Asteraceae were examined. *Capsicum frutescens*, *C. annum*, and *Datura arborea* of the Solanaceae exhibited mainly calcium oxalate crystals in the form of druses, styloids, crystal sand and prismatic crystals. In the seed coats of *Phaseolus vulgaris*, the prismatic and kink crystals observed may supplement the protective role of the seed coat for the developing embryo. Druse crystals were observed in *Iresine herbstii*, *Alternanthera ficoidea* "versicolor", and *A. ficoidea* "amoena". Results from this study may not only aid in taxonomical classification of plants, but may also be useful indicators of the physical and chemical conditions of the cell environment the crystals were formed. Knowing the abundance of crystals in different parts of the plant body especially the edible and medicinal plants may guide consumers as to the plant part and recommended age of the leaf to be used

P1513. Phylogenetic reconstruction of *Croton* section *Cyclostigma* reveals that it is a polyphyletic group

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Croton sect. *Cyclostigma* form one of the most distinct groups within the genus *Croton*. Members of *Croton* occupy a great diversity of habitats, from arid vegetation to lowland and montane forests and are important colonizers of disturbed sites. *Croton* sect. *Cyclostigma* represents one of the few arborescent lineages within a predominantly shrubby genus. It is also distinct from many other *Croton* lineages by its ecological preference for more humid habitats, especially montane cloud forests and riversides. Several species in the section are commonly known as "dragon's blood" due to the presence of blood-red latex, which is widely used for medicinal purposes in several South American countries. The section as defined by Webster consists of 62 species centered in the Neotropics, but with some disjunct members in Africa and Madagascar. Based on ITS and chloroplast data this work shows that section *Cyclostigma* is not monophyletic, however a well supported "core *Cyclostigma* clade" is identified. The relative

phylogenetic position of the section within the genus and the amphiatlantic distribution of the section is evaluated using a broader *Croton* molecular data set.

P1514. An Endemism of the Western of Lycian Toros (Babadag/Denizli) in Turkey

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The study area lies within 42° 00, 46° 60 north parallels and 28° 30, 29° 29 east longitudes. It is in the C2 grid square, according to the system adopted by Davis. Babadag is named as a Lycian Toros which is as a part of the western of Toros Mountain by Regel. The elevation is 100 meters at Karacasu on the western of study area. The eastern is Cankurtaran village, 1000 meters. The marble which originated from Baba hill is undergoing to Dikmen in the south of the study area. These series are covered with schist at the surface in the Gökbel valey, Tatarpinar and Yamaardic hill. In the study area, There are 1066 taxa 163 of which are endemic species 15,1%. Six of them are vulnerable plants according to red data list for the Flora of Turkey. Endemic taxa occupie between 500 and 2300m. They can be found calcareous rocks and in forest or cultivated lands. Most of them in the study area occupie calcareous rocks located in asilvatic belt at the elevation of 1750-2308m although some endemic taxa develops on phyll rocks. Evran hill, Karababa, Akbaba and Baba hills are the most important places for endemic taxa.

P1515. Trapping Device Evolution of Predatious Fungi: Evidence from Ribosome DNA and Multiprotein Sequences

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Nematode-trapping fungi produce special structures to capture nematodes. We performed a comprehensive phylogenetic analysis of predacious fungi to study the evolutionary relationships of trapping devices by using sequences from three protein-coding genes (RBP2, EF1- , & -tublin) and rDNA ITS region. Phylogenetic trees resulted from Maximum-likelihood analysis suggested that there is a strong phylogenetic component for the evolution of trapping devices. We proposed an evolution trend for the trapping devices based on both our phylogenetic tree and morphological characters. (i) The simple sessile knob-like special cells were assumed to be the ancestor of trapping devices; (ii) The simple sessile knob-like special cells were assumed to be the ancestor of trapping devices; (iii) Non-adhesive trap (constricting ring) was separated earlier from the adhesive traps (knob, stalked knob, hyphal branch, non-constricting ring and network); (iii) Among the adhesive traps, network was differentiated from the other traps; (iv) Then sessile knobs evolved into stalked knobs or non-constricting rings by the adhesive cells coiling or adhesive branches.

P1516. Distribution patterns and biodiversity in species of the genus Cousinia (sect. Cynaroideae)

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The *Cousinia* , with about 240 species, after *Astragalus* is the largest genus in the Flora of Iran. Nearly all of its species are found in arid zones, mostly in the Irano-Turanian region. A majority of the *Cousinia* species has a limited distribution and grows in small local areas. In the last researches done in 1972 and 1979 by Rechinger it led to dividing the *Cousinia* into 53 sections. The largest section is the sect. *Cynaroideae* with 88 species, of which 55 species are grown in Iran. The *Cynaroideae* section has the most morphologic variety among the genus. Recently we described 14 species from Iran. Based of our research this section includes 88 species in the world. 73 species of *Cynaroideae* were identified for Iran, of which 59 were endemic. After Iran, Iraq and Turkey have the biggest number on endemic species belonging to this section. The growth of 73 of the 88 species of *Cynaroideae* within Iranian borders is a testimony to the fact that Iran is only center of genetic diversity of the section.

P1517. Aponogeton satarensis, Trithuria konkanensis, Camporrhiza indica: living witnesses for the Wagner's theory of Continental drift

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During past two decades, I described more than 20 species of flowering plants from Western Ghats which is among eight hottest hot spots in the world. Three monocot species *Aponogeton satarensis* (Aponogetonaceae), *Camporrhiza indica* (Colchicaceae), *Trithuria konkanensis* (Hydatellaceae), are considered significant from biogeography standpoint. Cytogenetical investigations on *A. satarensis* and *A. decaryi* showed a close genetic relationship supporting the relation of Indian subcontinent to Madagascar. Morphological and cytogenetical investigations revealed a close relationship between *Camporrhiza indica* and *Iphigenia* species. An occurrence of the *Camporrhiza strumorsa*, the only other species of the genus in South Africa establishes relation of South Africa to Indian subcontinent. Discovery of *Trithuria konkanensis* of family Hydatellaceae has established the relation of Indian subcontinent to that of Australia. These three newly described monocot species are the living witnesses to support the Wagner's theory of continental drift.

P1518. Diversidade florística em ambientes distintos no Parque Nacional Vale do Catimbau, PE, Brasil.

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Universidade Federal Rural de Pernambuco, Recife, Brazil.

O Parque é considerado uma área de extrema importância biológica por apresentar vários ambientes, com diferentes tipos vegetacionais e uma flora bastante peculiar. Foram reconhecidos quatro tipos vegetacionais: rupestre; floresta úmida; arbustiva perenifolia; e caducifolia espinhosa (Caatinga). A flora vascular está formada por 370 espécies, distribuídas em 78 famílias, sendo 34 monocotiledóneas e as demais, dicotiledóneas. A família melhor representada em número de espécies foi Leguminosae com 59 espécies, sendo 25 Caesalpinioideae, 11 Mimosoideae e 23 Papilionoideae; seguidas de Euphorbiaceae (18), Asteraceae (11), Myrtaceae (10), Convolvulaceae (9), Malvaceae e Rubiaceae com 8. Os gêneros mais diversos foram *Croton* (Euphorbiaceae) 11 espécies, *Chamaecrista* (10) e *Senna* (7). Os ambientes com flora mais diversificada foram os rupestes e vegetação arbustiva perenifolia.

P1519. Floral diversity of African Marantaceae

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The Marantaceae (31/530) are a pantropical family of herbaceous monocots characterised by a highly derived pollination mechanism. An explosive movement of the style activated by the contact of the pollinator with a "trigger" guarantees the precise pollen transfer. Studies on American and Asian Marantaceae have shown a variety of adaptations to different pollinators based on diversity in floral morphology but nothing is yet known about the pollination systems and the correlated floral diversity in the African species. This, however, would be of special interest as Africa is regarded as the primary centre of diversity. African Marantaceae (10/33) form phylogenetically two mayor clades (basal and advanced) and three isolated genera. To reconstruct adaptive radiation in Marantaceae, we started a project dealing with the evolution of African species including both molecular and ecological aspects. The poster presents the first photographic overview of the existing floral diversity with emphasis on the diversity in "trigger" morphology including first inferences about their phylogeny and pollination.

P1520. Molecular systematics of Martyniaceae using the ITS region of nuclear ribosomal DNA

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Acceptance of the group Martyniaceae as a valid family has long been questioned. Often placed in the Pedaliaceae, the Martyniaceae have been allied to numerous other families, including the Gesneriaceae, Bignoniaceae, Acanthaceae, and Verbenaceae. Internal relationships within the Martyniaceae have not been explored using molecular data. For this study, sequences

of nuclear ribosomal DNA from the Internal Transcribed Spacer (ITS) region were used to examine these relationships. Several samples from eight families were included (Acanthaceae, Bignoniaceae, Gesneriaceae, Lamiaceae, Lentibulariaceae, Martyniaceae, Pedaliaceae, and Verbenaceae). Within the Martyniaceae, all four genera (*Craniolaria*, *Ibicella*, *Martynia*, *Proboscidea*) were sampled. The largest genus, the genus *Proboscidea*, was emphasized. The results of this study indicate that Martyniaceae is a distinct lineage. Within the Martyniaceae, the South American genera *Ibicella* and *Craniolaria* clustered, as did the North American genera *Proboscidea* and *Martynia*. Within *Proboscidea*, acceptance of three infrageneric groups is supported.

P1521. Biosystematic of African Celtis

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Celtidaceae, formerly Ulmaceae (Takahashi 1989) comprise ca. 180 species, classified in 9 genera, distributed in the Northern hemisphere and in Africa. *Celtis* L. is one of the most widely distributed tree genera in the world. In Africa 12 species are known: Some aims of this study are a revision of African *Celtis*, preparation of a conspectus of all *Celtis* species, producing an overall molecular phylogenetic hypothesis for Celtidaceae, and a phenology of *Celtis*. Methods used are loaning materials from different herbaria, study of macro-morphological and micro-morphological characters and observing phenology to understand seasonal ecophysiological response in *Celtis* by field observations in Wageningen Botanical Gardens, Molecular work will clarify relationships among species.

Two new species are described: *Celtis australiensis* Sattarian and *Celtis madagascariensis* Sattarian.

Endocarp characters proved to be important characters in African *Celtis*, Sunken area aperture in pollen grain as a main character in *Celtis* spp., all African *Celtis* pollen grains have been described.

P1522. A regional vascular plants catalogue from eastern Venezuela: Monagas State

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¹Universidad Central de Venezuela, Caracas, Venezuela, ²Universidad de Oriente, Monagas, Venezuela.

Venezuela is known as a megadiverse country, with 17.000 different plant species.

Monagas state (28.900 km²) in western Venezuela, within the Cordillera de la Costa as a northern boundary, the Orinoco river to the south, Venezuelan Llanos to the west and the Orinoco Delta to the east, reaches from 0 to 2600 masl. The state comprises ecosystems from mangrove formations, shrubby and open savannas, deciduous and humid forests, *Mauritia* palm swamps, wetlands, gallery forests, *Curatella* shrublands locally known as chaparrales and thorn scrubs. This is a poorly collected region. A check list made by the '80 estimated to 1.676 species. Today, 1.333 species have been counted only for the Llanos formation below 400 masl. A survey of plants previous to the vascular flora of the state is in course. Preliminary results of the plant diversity of the state reaches 204 families, 1105 genres, and 2566 species.

Specimens are deposited at CAR, GUYN, IRBR, MY, MYF, UOJ and PORT herbaria.

P1523. A regional vascular plants catalogue from eastern Venezuela: Monagas State

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¹Universidad Central de Venezuela, Caracas, Venezuela, ²Universidad de Oriente, Nucleo Monagas, Maturin, Venezuela.

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state is in course. Preliminary results of the plant diversity of the state reaches 204 families, 1105 genres, and 2566 species.

Specimens are deposited at CAR, GUYN, IRBR, MY, MYF, UOJ and PORT herbaria.

P1524. Morphological approach on the Vitaceae phylogeny

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Vitaceae are a family of mainly woody climbers characterized by the presence of leaf-opposed tendrils or inflorescences but with numerous exceptions. Floral differences and especially vegetative differences have historically been used to establish generic limits. However the relationships between genera have been proved difficult to establish.

This family is distributed worldwide and includes about 700 species parting in 14 genera. *Vitis*, *Ampelopsis* and *Parthenocissus* are mostly restricted to temperate areas. *Vitis* is concentrated in the northern hemisphere. *Parthenocissus* and *Ampelopsis* are Asian and American genera. The remaining genera have predominantly tropical and sub-tropical distributions. I have focused on morphological characters to try to achieve a morphological phylogenetic reconstruction of the family. Furthermore I studied the delimitation of the genera.

P1525. *Myxochlamys mullerensis* (Zingiberaceae), a new genus from Kalimantan, Indonesia

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A new genus and species, *Myxochlamys mullerensis* (Zingiberaceae) is described from the Muller Range, Central Kalimantan, Indonesia. The inflorescence is terminal on a leafy shoot with few leaves arranged in a tuft, and filled with plenty of mucilage inside bracts. The bracts are free to the base, spirally arranged, densely imbricate and subtending a single flower. The flower is similar to that of *Boesenbergia* but the anther is versatile with two long spurs at the base of thecae. The versatile anthers are known in five genera in the family so far: *Camptandra*, *Cautleya*, *Curcuma*, *Paracautleya*, and *Roscoea*. However, phylogenetic analysis using ITS sequences suggested the new plant is more closely related to *Scaphochlamys* and *Distichochlamys*, although the clade was not so strongly supported. Morphologically, however, *Myxochlamys* differs from these two genera by having entire and concave labellum and versatile anther with two long spurs.

P1526. Modelling the potential distribution of plant species in Colombia using Mahalanobis distances

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This work introduces a multivariate methodology for modelling the distribution of plant species in Colombia by means of integrating GIS with multivariate statistics. Predictive models represent an important tool to better understand the factors that control species distributions. Many of these have been developed in temperate areas, however poorly sampled tropical regions, where the highest biodiversity areas remain and models might be of major value, have hardly ever been considered. Primary inventory data exist in the best of cases as georeferenced coordinates from localities where specimens have been collected. There is rarely data which indicates absence or abundance of species. However, most of the current modelling approaches need the existence of both presence and absence data, and many of them are based only on biological tolerance to climate. The methodology here illustrated represents a step towards the achievement of an efficient use of the data already available in Colombia by providing a technique for modelling potential species distribution that utilizes only presence data based on Mahalanobis distances applied to two species of *Palicourea*.

P1527. The Middle Devonian proto-ovule *Runcaria heinzlinii* Stockmans 1968

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Contrasting hypotheses about the single/multiple, saltational/gradual origin of the seed habit, and identification of closest relatives to seed plants among progymnosperms are matters of considerable debate. We have identified a middle Givetian (Middle Devonian; approximately 385 million years ago) proto-ovule that pushes back the earliest evidence of seeds by about 20 million years. *Runcaria heinzelinii* Stockmans 1968 is a small radially symmetrical proto-ovule surrounded by a cupule. It possesses an indehiscent megasporangium bearing a distal columnar extension, differing from that of all early preovules by its extensive length and closed apex protruding above a multilobed spiraling integument. This morphology suggests a reproductive mechanism that combines anemophilous pollination and dissolution of sporangial cells to allow fertilization. *Runcaria* displays a highly derived morphology compared to that of progymnosperm sporangia which sheds new light on the order of character addition leading to the seed. It suggests that the seed syndrome originated earlier than the middle Givetian, when all known progymnosperms were aneurophytales.

P1528. Comparison of two morphological *Stratiotes aloides* forms

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Stratiotes aloides forms in Wielkie Słosineckie Lake situated in Poland were examined. The investigation was aimed at demonstrating differences between the floating form of *S. aloides* and the submerged one. 40 specimens and 909 leaves of *S. aloides* were examined. The following plant attributes were examined: width, length, sex, number of generative and vegetative organs, dry mass of whole plants and their organs. The examined attributes of leaves: leaf apex angle; leaf width measured 3 cm from the base, 10 cm from the base and 10 cm from the top; leaf area; cellular wall thickness; number of vascular bundles, and number of chloroplasts. The leaf area was determined with an image analyzer Met-Ilo 8. Leaf cells were examined with the use of a confocal and optical microscope. Water and bottom deposits from the places of specimen collection were analysed. The results confirm the occurrence of the two morphological forms of *S. aloides* in Wielkie Słosineckie Lake as well as the morphological and anatomical differentiation between them.

P1529. Male and female gametes from some woody plants: isolation and characterization

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Isolated viable gametes are prerequisite for *in vitro* fertilization and available for cytological studies. The isolation and manipulation of viable gametes has been technically challenging for woody plants. By enzymatic-osmotic shock method, we have successfully isolated viable gametes from several woody species.

1. Sperms formation and positional shift among components of the male germ unit were observed in *Paeonia suffruticosa* after inoculated in a BK medium with 12.5% sucrose. After enzymatic digestion and osmotic shock, sperms were released from a subapical pore of pollen tubes and were FDA positive
2. Embryo sacs/egg apparatuses were isolated from *Populus euphratica* by enzymatic digestion and squash. Stereofeatures of fertilization were observed during isolation. The cutinized covers of nucellus and interguments showed the structural obstacles to the isolation of egg cell.
3. Archegonia of *Pinus tabulaeformis* were isolated from enzymatic solution via an osmotic shock. Egg cells were released at 18h, and were FDA positive. In the protein inclusions that are bounded by a double membrane, there are trapped plastids, mitochondria and polyribosomes.

P1530. Diversity of Stingless bee (*Trigona collina* Smith) 's floras in Dicliduous Forest of Doi Mon Long at Queen Sirikit Botanic Garden, Chiang Mai, Thailand by Pollen Load analysis

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Abstract

Changing of environment effects the balance of ecosystem which leads to the decrease of Stingless bee (*Trigona collina* Smith) population in Thailand. This study could clarify the food plant species for Stingless bee in the deciduous forest of Queen Sirikit Botanic Garden, Chiang Mai province. The result will be useful for the conservation management of this bee species. Following the acetolysis technique, 20 plant species were recorded as food plants of the bees according to their external morphology. These are in the family: Mimosaceae, Asteraceae, Loranthaceae, Euphorbiaceae, Sapindaceae and Juglandaceae. The Pollen number 2 (P2) was *Tridax procumbens* L., (P3) was *Croton oblongifolius* Roxb., (P11) was *Litchi chinensis* Sorm., (P16) was *Mimosa pigra* L., (P18) was *Engelhardtia spicata* Bl. and (P20) was *Helixanthera* sp. The higher abundance of the melliferous plant species was recorded between January-March, whereas fewer supply was found during July-September. Moreover, the number of food plant species was not positively correlated with the number of flowering plants. This phenomena exhibited the preference behavior of the bee.

P1531. The foliar anatomy of forest climax tree species in relation to forest development in Orange Kloof, Table Mountain, South Africa.

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This investigation deals with the foliar features and functional anatomy of four forest climax tree species growing in a forest and the adjoining fynbos protected from disturbance for over 50 years. These foliar features, anatomical and morphological, were investigated within the forest interior, forest margin and the adjoining fynbos, and between the stream and non-stream habitats on Table Mountain. The aim of the investigation was to understand the functional significance of foliar anatomy in forest ecotonal dynamics. The foliage of the forest climax species displayed a distinctively thin cuticle. Results suggest that the forest climax species predominantly possess foliage acclimated to one set of environmental conditions - the forest interior. The predominant lack of significance differences between leaf characters of any given tree species in the forest climax and in the forest margin suggest ecological similarities between these environments. It appears that a lack of adaptive ability both anatomically and morphologically restricts the colonisation of the forest-adjointing fynbos by forest climatic tree species.

P1532. Application of Molecular Markers on Nomenclature for Ornamental Peach

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Ornamental peach (*Prunus persica* (L.) Batsch) is a popular ornamental plant with many published and new taxa. Unfortunately, its nomenclature and classification in the literature are contradictory and confusing, which limited further breeding and development. Molecular markers have been increasingly applied for distinguishing different taxa by producing robust, reliable and objective data for taxon descriptions. DNA fingerprinting library of all 51 available ornamental peach taxa has been established by using six EcoRI/MseI primer combinations of AFLP data. Among the 51 collected taxa, 32 cultivars derived from two species are legitimate taxa. These molecular data, particularly unique bands and band patterns of a taxon, could apply for identifying and supporting the legitimate taxon of ornamental peach. Application of AFLP data on nomenclature provides an example to aid future naming of ornamental taxa based on *International Code of Nomenclature for Cultivated Plants*. To include molecular data into the articles of ICNCP, recommendation provisions based on ornamental peach AFLP analysis were proposed.

P1533. Morphological and genetic diversity of endemic genus *Boninia* in the Bonin (Ogasawara) Islands, Japan

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Boninia is one of two endemic genera found on Bonin (Ogasawara) islands located in the northwestern Pacific Ocean.

Boninia glabra, *B. grisea* and *B. grisea* var. *crassifolia* have been described, occupying a diversity of mesic to xeric habitats from sea cliffs to mountain ridge above 900 meters.

Morphological analysis based on 19 morphological characters was performed in total of eight populations of three taxa. The PCA analysis and the phenogram of cluster analysis revealed existence of morphological subgroups within *B. grisea* populations, on the other hand, each of *B. glabra* and *B. grisea* var. *crassifolia* formed a coherent cluster.

Genetic diversities within and among populations were examined using AFLPs. Analysis of molecular variance showed that 72.8% of variability was partitioned among individuals within populations. In the NJ analysis, there was hardly any structure and none of taxa formed distinct groups.

Morphological and genetic diversification within *B. grisea* could suggest incipient form of adaptive radiation, and disagreement of morphological and genetic boundaries of the other two taxa implies recent divergence.

P1534. The Database of Vascular Plants of Sichuan and Chongqing in the southwest of China

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There are more than 11,000 species of Vascular plants in Sichuan province and Chongqing City (VPSC) in the southwest of China. One of the famous biodiversity "hot-spot" areas- Hengduan Mountains is located in Western Sichuan and Eastern Xizang. A database of these species has been set up in recent years. VPSC is a part of the biodiversity information system project in the southwest of China supported by Chinese Academy of Sciences. VPSC provides an online information search. The species names and associated information including Chinese names, local names, images, distributions, resource use and related publications will be available.

In the database, the species names are based on the recently publications, such as Flora of China, especially monographs of revision. Some important synonymous in Flora Reipublicae Popularis Sinicae and Flora Sichuanica are cited.

Full Chinese information has been available in the database, and full English information will be available in the recent months.

P1535. Miquel's new taxa of the flowering plants described from Japan in Prolusio Florae Japonicae and some other works

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Dr. F. A. W. Miquel (1811--1871) studied the collections of Japanese plants collected by Phillip Franz von Siebold and his successors kept in the Rijksherbarium, Leiden (L). He described many new taxa based on those collections in *Prolusio Florae Japonicae* in his *Annales Musei Botanici Lugduno-Batavi*, and in other works between 1850 and 1868, but did not typify them in the present sense, since the type concept was not practice at that time. In studying the Japanese flora, there is a need to clarify Miquel's taxa and to lectotypify them. The labels of the collections studied by Miquel usually give the locality only as "Japonia," and the name of the collector. The specimens also bear Miquel's determination. Additionally, some specimens have labels with the original information in Japanese or other languages, which are useful for identifying the cited specimens in Miquel's articles.

As aid to those studying the Japanese flora, we have prepared a list of the taxa from Japan described by Miquel and have lectotypified a number of names for which a type specimen was not previously designated.

P1536. Sexual systems of tree species in two tropical forests of Brazil

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The distribution of sexual functions within and between individuals in plant populations are reproductive strategies that seem to be shaped by selective pressures such as the pollination ecology and the mating systems. The great majority of tree species is hermaphrodite, being generally followed by the dioecy and monoecy. The objective of this study is to characterize the distribution of sexual systems of tree species in 10ha. of a

Restinga forest and 10ha. of Cerrado forest in SE Brazil. Sexual systems were determined through the observation of morphological and functional male and female elements of flowers. The majority of the species studied were classified as hermaphrodite, being followed by dioecy and monoecy, what corroborates the patterns discussed above. Dioecious families recorded here were also recorded in other tropical environments, and generally presented small flowers. We found some evidences of androdioecy and ginodioecy, but a larger sample is necessary to draw any definitive conclusions.

P1537. Diversity Arrays Technology (DArT™): a promising DNA microarray-based comparative technique for molecular systematics and DNA barcoding

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Conventional techniques in molecular systematics typically involve time-consuming labor components that, while not prohibitive, limit the rate of scientific progress and pose a special challenge to comprehensive efforts like DNA barcoding. Developed to map variation within crop species, Diversity Arrays Technology (DArT™) rapidly identifies and genotypes hundreds of DNA polymorphisms that differentiate closely related organisms. Because DArT does not require prior sequence information, it is suitable for non-model organism research, potentially bridging the gap between genome-era biotechnology and studies of the natural world. To explore DArT as a tool for systematics and DNA barcoding, we commissioned Diversity Arrays Technology Pty Ltd to generate DArT data for two test sets of ferns and bryophytes. We examine the capacity of DArT data to resolve relationships, and we test DArT against specific challenges like hybridization and polyploidy, which seem likely to complicate barcoding. We discuss the potential applications of DArT in different arenas of plant research and propose a strategy for using DArT as a complementary tool for DNA barcoding.

P1538. Phylogenetic relationship of the Collemataceae(Lecanorales) family inferred from mSSU and nLSU rDNA sequences

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Collemataceae is a family of lichenized ascomycetes comprising the genera *Collema* F.H. Wigg., and *Leptogium* (Ach.) Gray, both with septate spores, and *Physma* A. Massal., *Staurolemma* Körb., and *Ramalodium* Nyl., which all have simple spores. The generic concept in this family is based on the presence or absence of a eucortex. *Collema* is divided according to the nature of the proper exciple, spore shape and thallus size. *Leptogium* is classified by, presence/absence of tomentum and lower cortex, spore shape and thallus anatomy. However, phylogenetic studies of Lecanorales suggest that *Collema* and *Leptogium* are not monophyletic. In order to evaluate relationships and re-circumscribe the genera in this family using monophyly as the grouping criterion, we have conducted phylogenetic analyses on mtSSU and nuLSU rDNA sequences from species representing the morphological diversity recorded for these two genera. Members of the Placynthiaceae and Pannariaceae were used as outgroup taxa for this study. Our results suggest that the early evolution of the *Collema*-*Leptogium* complex was associated with differential ecological requirements and thallus size.

P1539. Report of a new *Lepanthes* from Colombia

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It is presented the report of *Lepanthes magnifica* Luer from Colombia, this species was known solely of the Ecuadorian north-east, but recently it was collected in the south-west of Colombia in Nariño Department between 1100 and 1300 meters of altitude. *Lepanthes* is the fourth genus more diverse from Colombian with 277 species; most species inhabit cloud forests where the temperature is low and humidity high. A significant number of species are limited in distribution, resulting in high levels of endemism, near 40 % of species are endemic of Colombia, but due to the present situation of the Andean forests most of the

populations they are very low, therefore many of the species can be in extinction danger. Few it is known systematics, ecology, reproductive Biology and taxonomy of *Lepanthes*.

P1540. Phylogenetic analysis of *Calathea* Subgenus *Microcephalum*

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The family Marantaceae is composed for 31 genera and near 530 species. The genus with the greatest number of species in the family is *Calathea*, with around 300 species. *Calathea* in the monograph for the family consist of four subgeneras: *Eucalthea*, *Macropus*, *Pseudophrynium* and *Microcephalum*. This subgenus is conformed by the smallest species of *Calathea*, one of them have an extensively distributed in the Neotrópico (*Calathea micans*) and the remaining seven species occurring in (*C. compacta*, *C. curaraya*, *C. dicephala*, *C. fucata*, *C. microcephala*, *C. pearcei*, *C. undulata*). The most relevant morphological character used to distinguish *Microcephalum* are small inflorescences with few bracts.

The goal of this work is to conduct a cladistics analysis based on morphological, anatomical and molecular (*matK*, *trnL-F* y *trnK 3'*) data in order to better understand the evolution and classification of the members in Subgenus *Microcephalum* and propose a phylogenetic hypothesis for this subgenus inside *Calathea*.

P1541. Show me the stone cells and I can see where you picked that *Dionysia*!

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The phylogeny of the genus *Dionysia* (Primulaceae) based on sequence data from ca 50 species differs from earlier classifications. The genus *Dionysia* (mostly tufts or cushion-forming subshrubs) grows in mountains in the Middle East. When cladograms are projected onto a distribution map, a distinct eastern-western pattern can be seen.

Many morphological characters in *Dionysia* are size-dependent, and thus a reduction in size in several clades have resulted in homologies. Leaf stone cells (sclereids) is a size-independent character that contains much informative variation. Our study indicates that only for two types of stone cells have parallel evolution produced forms that look really similar. Other types of stone cells are limited to single clades. Bundles of terminal stone cells are characteristic of one western clade, and diffused stone cells appear only in eastern species, with one exception.

Leaf stone cells provide phylogenetic information and are very useful when trying to determine the position of a newly discovered species of *Dionysia*

P1542. Haustorium type, endophytic system form and vegetative reproduction in four *Scurrula* L. species from Nepal

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Epicortical roots (ER) occurred in all *Scurrula* species regardless of host or elevation. Primary and secondary haustoria evoked a wood-rose response by host. Wood-rose form varied between and within species. Primary haustorium structural differences allowed classification into (1) flanging endophyte, (2) flanging endophyte with radial shafts, and, (3) sinker endophyte. *Scurrula elata* and *S. gracilifolia* differed in haustorial form on different hosts, and *S. elata* had the widest host range due to its specific haustorial and endophytic system plasticity. Epicortical roots of *Scurrula* resembled those of other Old World species in origin, morphology and branching pattern, but differed from those of New World species. The formation of secondary haustoria and root-borne shoots by ERs was common, but their frequency varied depending on the age of the mistletoe and the length and vigour of epicortical roots.

P1542a. Systematics of *Lambertia* Sm. (Proteaceae)

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Lambertia Sm., (Subfamily Grevilleoideae, Tribe Macadamieae, Subtribe Lambertiinae) is an Australian genus of 10 species, with

nine species in the south-west of Western Australia and the remaining one in eastern Australia. It includes three critically endangered species (*L. echinata* R.Br., *L. fairallii* Keighery and *L. orbifolia* C.A.Gardner) and two poorly known taxa (*L. multiflora* Lindl. and *L. rariflora* Meisn.). Despite limited recent taxonomic and genetic work a number of important taxonomic, evolutionary and biographical questions remain. This project aims to resolve taxonomic issues with particular reference to the threatened taxa and to produce a phylogeny of the genus. The relationships between sub-species, especially those which are separated geographically or for which preliminary molecular analysis indicates differences, will be addressed and the monophyly of *Lambertia* and its relationships with putative sister taxa will be investigated.

P1543. *Ranunculus glacialis* and *Ranunculus alpestris*: sexual reproduction in a high mountain environment

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The duration of the growing season is the most important factor limiting plant life in high alpine and arctic habitats. Depending on relief and snow accumulation in winter, 3-5 months in the alpine belt and 1-3 months in the nival belt can be used for growth and reproduction in the mountains of the temperate zone. In a survey on different mountain species in the alpine and subnival zone, *R. alpestris* and *R. glacialis* showed a particularly rapid and efficient seed production. Anthesis started 10 to 14 days after snowmelt. Stigmas were receptive as soon as flower buds opened so that seed development could start immediately after pollination. In *R. alpestris* fruits began to disperse already 24 days after anthesis, in *R. glacialis* the period of seed development ranged between 28 and 36 days. Seed set was around 53% in *R. alpestris* but attained up to 82% in *R. glacialis*. In both species, flower preformation for the following year coincides with the period of seed development. After fruit dispersal, leaves soon become senescent and die back. Thus, to complete the reproductive cycle, a growing period of 6-7 weeks per year is sufficient for both species.

P1544. Life strategy of the subnival species of *Cerastium* (Caryophyllaceae) from the Central Greater Caucasus and the Alps

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Life history and reproductive biology (number and sizes of flowers, ovules and seeds, seed set, embryology) have been studied in *Cerastium kazbek* from the Caucasus and *C. uniflorum* from the Alps. They are perennial herbs. *C. kazbek* reaches upper distribution limit of flowering plants in the Caucasus, 4000 m a.s.l., and is almost absent below 3000 m. *C. uniflorum* grows between 1900-3400 m a.s.l. Flower primordia are formed in early spring under snow cover. After snow melting flower buds start to develop. The first flowers open in the end of June in *C. kazbek* and end of July in *C. uniflorum*. Flowering is not simultaneous in *C. kazbek* and new buds are formed basipetally. The first fruits mature in August-September. Seed dispersal continues until October, when new snow cover ceases vegetative cycle. *C. kazbek* enters winter bearing fruits, flowers and buds in different developmental stages. The aboveground parts of the plant are frozen except basal leaves, which stay green during winter.

P1545. Sexual and clonal reproduction in the alpine *Geum reptans*: effects on demography, genetic variation and population differentiation

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Spatially isolated populations are more the rule than the exception in alpine plant life. Selection has resulted in a high frequency of long-lived species that rely on clonal growth for population dynamics. We studied the importance of sexual vs. clonal reproduction in a pioneer plant from glacier foreland for its local and spatial dynamics. A main focus was to test whether variation in reproductive behavior depends on population origin from successional stage and elevation. With RAPD-PCR we measured genetic diversity, differentiation and gene flow among populations with distances ranging from 0.2 to 208 km. In field and greenhouse experiments we tested how population origin and environment

affect reproduction. Matrix modeling suggests that in *Geum reptans* sexual reproduction is equally important for population dynamics as clonal reproduction. Genetic diversity in populations was high and irrespective of population origin, populations were moderately differentiated. Results from experiments suggest limited adaptation to contrasting habitats and a high potential to maintain growth and reproduction under a large range of habitat conditions.

P1546. Breeding systems and rarity: pollination requirements and micro- and macro-distribution patterns in New Zealand forget-me-nots (*Myosotis* spp).

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The alpine forget-me-nots (*Myosotis* spp) of New Zealand are all self-compatible, but fall into two breeding types - autonomous selfers, and pollinator-requiring species. This separation has apparently led to fundamental differences in distribution patterns. The pollinator-dependent species are all range-restricted, in some cases to the state of being point-endemics. These species tend to show poor reproductive success at low densities (i.e. they suffer from Allee effects), while the selfers are often in small sparse populations, but are more widespread and in these species seed set is not affected by the density of the population. We show that one character - herkogamy (the distance between anthers and stigma) accurately predicts geographic range of 33 species. Thus, our work demonstrates the importance of knowledge of breeding systems of rare plants for understanding conservation status and distribution pattern.

P1547. Breeding system of high mountain plant species in the European Alps

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In our study we investigated four alpine species (*Saxifraga androsacea*, *S.caesia*, *S.moschata*, *Ranunculus alpestris*) and three nival species (*Saxifraga bryoides*, *Ranunculus glacialis*, *Cerastium uniflorum*). All *Saxifraga* species showed a mixed mating system with the highest reproductive success after natural pollination (mean S/O ratio 0.5-0.6). After natural selfing, the S/O ratio ranged between 0.1 and 0.4, but artificial selfing could markedly increase seed set. Apomixis seemed to be irrelevant in these species.

C.uniflorum turned out to be a clear outcrosser (mean S/O ratio 0.6) yielding no seeds by natural selfing and apomixis. The efficiency of artificial selfing was with 15% rather low, signifying a high degree of self incompatibility. *R.glacialis* attained S/O ratios of up to 0.8 after outcrossing, but only up to 0.12 after selfing and apomixis. *R.alpestris* showed a rather flexible breeding system: natural crossing and natural selfing led to about the same reproductive success (S/O ratio about 0.4) and about 20 percent of flowers performed apomixis. In total it could be shown that all species except for *R.alpestris* strongly depend on pollination by insects.

P1548. Conditions affecting recruitment of seedling of *Espeletia grandiflora* in Parque Nacional Natural Chingaza, (Colombia).

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ABSTRACT

In this study two sites were sampled to evaluate conditions influencing recruitment of seedling of *Espeletia* in Parque Nacional Natural Chingaza (Cundinamarca, Colombia). As results, we could find more microsites on second sampling, down on slope's gradient. This can be explained because of water streams that run down by the slope, carry seeds and deposit them at the end of the slope or in places with smaller slope. Most of sites for implantation corresponded to free-spaces in *Calamagrostis effusa* matrix occasioned by falling of *E. grandiflora*'s plants and necromass present, where seed are trapped and deposited. It was also found that size of microsites is directly related with number of seedling which get implanted. Another factor that seems to play an important role is distance to mother plant, being more frequent seedling in places near to source, given that mother plants modify microenvironmental conditions generating special conditions which

favor implantation because seeds are covered and protected from low temperatures.

Then, implantation is determined by slope, diversity of microsites, and distance to plant mother.

P1549. Life history traits and population dynamic of *Saxifraga aizoides* in an alpine succession

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Saxifraga aizoides is a pioneer species on the earliest stages of deglaciated terrain (moraines of 1971) on the foreland of the Rotmoos glacier (Central Alps, Tyrol, Austria). *Saxifraga* can be regarded as target species for investigating both the colonization mechanisms as well as the population dynamics. The ongoing project (2003-2007) is analysing the life history traits of this species along three different moraines. This poster will show the first results of soil seed bank, seed rain, germination ability and growth studies of *Saxifraga aizoides*.

20 permanent plots of 1 m² were established per moraine stage (moraines 1971, 1923, 1858 and one site beyond the terminal moraine) during the summer 2004. In each plot 5 soil samples were collected, a seed trap was installed, individuals of *Saxifraga* were marked and counted and several fruits were collected for germination experiments.

Saxifraga aizoides seems to have permanent soil seeds banks and to be well dispersed along the two young moraine stages. The regeneration of the population seems to be successfully on the 1971 moraine (612 seedlings) but not on the 1923 moraine (0 seedlings).

P1550. Reproductive biology of subalpine endemic *Minuartia nifensis* Mc Neill from Turkey

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The *Minuartia nifensis* species found in Turkey, is one of the 16 members which belong to the *Xeralsine* subsection of the *Minuartia* section of the *Caryophyllaceae* family. The *Minuartia nifensis* species was first discovered on the Nif mountain peak by Reino Alava in the year 1966. In 1969, Mc Neill who had been doing research on the *Xeralsine* subsection, categorized this species as a distinct endemic one that can be found only on the Nif mountain (Davis 1988).

As indicated by John Mc Neill, the taxonomy of this subsection, of which *Minuartia nifensis* is a member, is extremely complicated and its area of existence within Turkey is still unclear. Mc Neill suspected that the reproductive biology of the group to which *Minuartia nifensis* belongs was somewhat unusual and wondered about the possibility of agamospermy.

This study has several goals. The first goal is to determine the population growth areas and estimate the number of individuals. The next one is to update the IUCN category of *Minuartia nifensis*, with the aid of the acquired data and to determine the reproductive biology. And the final goal is to develop specific conservation strategies.

P1551. Is there a female advantage in seed weight in gynodioecious Mountain Thyme along altitudinal gradients?

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The frequency of females of gynodioecious *Thymus praecox* increases along altitudinal gradients in the Alps, maybe caused by a female advantage in seed weight at higher altitudes. Two sources of variation in seed weight were detected: (1) allocation of resources within fruits (linear decrease in weight with increasing seed number) and (2) altitude (heavier seeds at higher elevations). A transplant experiment showed genotypic differentiation for heavier seeds at higher altitudes. This could allow selection to act on the sex ratio with a stronger female advantage at higher altitudes. Indeed, females of *T. praecox* provisioned seeds better than hermaphrodites, but the relative advantage was constant at different altitudes. Moreover, hermaphrodites set less and thus heavier seeds, while females set more but lighter seeds. This counteracted the postulated female advantage from seed provisioning. Hence, no correlation between female advantage and current sex ratios was found, and the importance of genotypic

differentiation in seed weight along altitudinal gradients for the maintenance of current sex ratios in Mountain Thyme is still not clear.

P1552. Genetic diversity in two glacier forelands in the Central Alps

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Glacier forelands offer the possibility to investigate the development of a newly formed ecosystem from the very beginning. So far little is known about the population dynamics of early settling species occurring there. The knowledge of genetic diversity within and among glacier foreland populations may contribute to a better understanding of the colonisation processes. *Saxifraga aizoides* is a dominant early colonising species, highly abundant within two parallel glacier valleys in the research area in the Oetzal (Central Alps, Tyrol, Austria). 16 populations of *Saxifraga aizoides* from different moraine stages (dated 1923, 1872, and 1858) and the adjacent valley slopes were exemplary investigated by an AFLP marker system. 209 fragments could be amplified, of which 92.8 % were polymorphic. The data were analysed using the AMOVA technique to test the variation within populations and among them as well as between the different glacier valleys. Furthermore, allocation procedures (AFLPOP) were performed to gain conclusive insights in the colonisation history.

P1553. Is there apospory in sunflower?

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The phenomenon of apospory is interested in selection and genetic aspects (Batygina, 1999); in relation to this some data about formation of additional embryo sacs [AES] in sunflower are presented.

The ovule is tenuinucellate, with one integument, integumentary tapetum [IT] and one embryo sac [ES] that develops according Polygonum-type.

The *Helianthus annuus* (line VIR116) was pollinated by *H. occidentalis*. Temporal fixations of ovule permit to reveal AES at different time after pollination (from 50 m. to 56 h.). The 83 ovule were investigated and the AES were found in 15 of them. The penetration of pollen tubes into the ovules was not occurred. Almost all AES units are similar to those in ES except antipodals. They are not arranged linearly and have smaller sizes, spherical form and dark stained cytoplasm. The AES are not surrounded by IT, have the irregular form and located behind ES, but do not adjoin to it.

The data allow supposing that AES, founded in VIR116, can be of aposporic origin.

P1554. Apogamy and genetic heterogeneity in genus *Allium* L. (Alliaceae L.)

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Polyembryony and connected with its apogamy is cause of genetic heterogeneity, since embryos arising in one seed (twins and triplets) have different inheritance (uniparental, biparental). Apogamy is rare phenomenon, because its reasons, origin and genesis of adventitious embryos are poorly understood. In genus *Allium* the both form of apogamy are found. Comparative cytoembryological investigation of ovule, embryo sac and adventitious embryos genesis in two species *Allium* with synergical (*A. schoenoprasum*) and antipodal (*A. ramosum*) apogamy is carried out. It was revealed that during embryo sac development the synergid (*A. schoenoprasum*) or antipod (*A. ramosum*) becomes egg-like. Development of zygotic and adventitious embryos is similar (Onograd-type). Owing to competition most of adventitious embryos degenerate in early stages. The frequency of polyembryony in mature seeds is 0,1 % in *A. schoenoprasum* and 6 % in *A. ramosum*. Also various deviations in embryo sac development in *A. ramosum* are found. Perhaps, the adventitious embryos are appeared in seed due to the interaction different factors (genetic, physiological, ecological etc.).

P1555. Sexual reproduction as a factor influencing population genetic structure in agamic complex of *Hieracium* subgenus *Pilosella*

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Significance of sexual reproduction in populations of facultative apomicts is investigated. Model system consisting of hexaploid apomictic *Hieracium bauhini*, tetraploid sexual *H. pilosella* (parent accessions), their natural hybrids, and their artificially generated hybrids is used. Crossing experiments between parental accessions were carried out to quantify apomixis, sexuality and haploid parthenogenesis in *H. bauhini*; 95.47% of progeny was produced by apomixis, 2.47% of progeny were BII hybrids, 0.41% were BIII hybrids, and 1.65% were trihaploids in this accession. Mating system of particular progeny was estimated by emasculation experiments. Almost all BII hybrids were sexual and almost all trihaploids were apomicts; some selection acts here. Mating system has not been estimated in BIII hybrid. Experimental backcrossing of artificial hybrids is planned to examine the role of such hybrids in hybridisations in natural populations. It is also planned to compare artificial hybrids (with known origin) with natural hybrids of unknown origin. This will enable us to detect processes that could contribute to the origin of hybrids in the field.

P1556. Apomictic Taxa in the Vegetation of Central Europe

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Predominantly agamosperous species account for more than 1/3 of Central Europe's Higher Plants.

The presence of apomictic taxa was evaluated in 200.000 phytosociological relevés. 10 ecological qualities (water supply; calcium & magnesium supply; nutrient supply; environment dynamics; hemeroby; frequency of stresstolerant species; frequency of salt tolerant species; altitude; vegetation height; frequency of annuals) were rated in 143 syntaxonomical alliances in a 5-step scale and correlated with the number of apomicts.

Corrected mean numbers of apomicts vary from 0 to 1.9 per relevé and alliance. Rang correlations revealed significant data only for the traits altitude (more apomicts than expected), water (less apomicts), and salt (less apomicts). Apomicts do not predominantly occur in habitats with under average competition. They do not have larger ranges than related amphimicts. No explanation can be presented for the above average numbers of apomicts in high altitude habitats. Absence of apomicts from the species-poor habitats with high values for the traits water and salt might be artifacts.

P1557. Evolution of new apomictic interspecific hybrids from a sexual diploid *Allium senescens* var. *minor*

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All known apomictic species in nature are polyploid. To understand evolution of an apomictic hexaploid species *Allium senescens* (AS), we focused on a diploid sexual species *A. senescens* var. *minor* (ASM). Two species are taxonomically very near and the seed setting of their interspecific hybrid is normal. We harvested seeds of ASM which were planted near with AS and tetraploid interspecific hybrids from a cross between ASM and AS. Among 192 seedlings of ASM, 16 plants (8%) are triploid and 2 plants (1%) are tetraploids. Result of karyotype suggests that triploids and tetraploids are not developed by ASM itself but developed by contribution of foreign genome. All 88 flowered diploid plants are sexual but 2 of 15 flowered triploid plants and 1 flowered tetraploid plant showed parthenogenetic embryogenesis. The shape of parthenogenetic embryo developed from egg cell or antipodal cell is same with that of AS. Triploid, tetraploid and pentaploid AS or ASM x AS interspecific hybrid could be developed naturally by open pollination of sexual plant with pollen from different ploidy plant. Through the process apomixis also delivered and expressed in polyploid plant.

P1558. Relevance of clonal propagation of perennial plants in the semiarid Valley of Zapotitlán, Puebla, México.M. D. García Suárez¹, J. L. Gómez Olivares², H. Serrano²;¹Biology Department, Universidad Autónoma Metropolitana Iztapalapa México, D.F., Mexico City 09340, Mexico, ²Dept. Health Sciences, Universidad Autónoma Metropolitana Iztapalapa México, D.F., Mexico City 09340, Mexico.

Plant survival in arid and semiarid environments represents a challenge due to harsh conditions. Vegetative reproduction operates as a safe strategy to perpetuate genotype. The clonal propagation of perennial plants was registered in 50 x 50 m permanent quadrants in the Zapotitlán Valley, in order to evaluate its efficiency and the different alternatives of vegetative propagation that these plants present. We found that the most common reproductive strategy was the formation of basal shoots some examples are: *Agave marmorata*, *Agave macroacantha*, *Tillandsia dasyliirifolia*, *Tillandsia califanii*, *Echinocactus platyacanthus* and *Ferocactus latispinus*; rhizomatous shoots *Hechtia aff. podantha*, *Agave marmorata*, *Agave macroacantha*; buds *Yucca periculosa*, *Coriphanta radians*; bulbils *Agave marmorata*, *Agave macroacantha*; cladodes *Opuntia sp.* For some of these species, vegetative propagation has proved to be more efficient than seed propagation, allowing their survival in this extreme environment.

P1559. Structural and biochemical aspects of the life strategy of *Colchicum autumnale* L.L. Franková¹, K. Bóka², O. Gašparíková¹, M. Pšenák³;¹Institute of Botany, SAS, Bratislava, Slovakia, ²ELTE University, Budapest, Hungary, ³Comenius University, Faculty of Pharmacy, Bratislava, Slovakia.

Colchicum autumnale is a clonal, hysteranthous geophyte. Starch is the main storage material of ripe corm. 2/3 of starch of mother corm is reutilized during autumnal developmental stage, supporting development of shoot and flowers when the net production of reduced carbon does not exist at all. This decline was paralleled by increase in total amyolytic activity, consisting mainly of -amylase, -amylase and -glucosidase activities. Sucrose, glucose and fructose were the only free sugars detected in the corms, with maximum level during winter season. Using native PAGE a specific protein complex was identified in all organs of the plant. The rest of starch was completely reallocated from the mother to daughter corm during the photosynthetically active stage. Structural analyses showed the presence of sclerenchymatic tissue in the protuberance, helping the new corm to penetrate through the soil. On the boundary of new corm and shoot a meristematic layer was recognized. This layer is later on changed to abscission zone, which initiates the dying back of above-ground part regularly at the end of annual life-cycle. Supported by grant VEGA No. 1/1275/04.

P1560. Clonality on narrow endemic and relict *Dianthus callizonus* Schott et Kotschy in Romanian Carpathians

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Dianthus callizonus (The Little Carnation of Piatra Craiului) has its distribution in alpine and subalpine areas in Piatra Craiului Massif from Romanian Carpathians, on fixed and semifixed calcareous scree, grasslands. Due to the geomorphological and climate characteristics of the Massif, *D. callizonus* is a rare, relict and endemic species; limited resources can be invested either for sexual or for vegetative reproduction. Being included in different plant associations and having modular construction, the growth strategy may be realized by differences in the spatial arrangement and size of shoots, producing buds on horizontal roots and plagiotropic shoots which annually renew the aerial shoot system, adaptively associated with the stressful conditions of the habitats. Its distribution and maintenance in Piatra Craiului Massif depend on its pronounced capacity to expend laterally for reaching more areas inside of a very fragmented habitat and to resist in the same area.

P1561. Natural regeneration system of *Cryptomeria japonica* in a natural forestK. Mishima¹, K. Takata¹, A. Makita², S. Sawada³;¹Akita Prefectural University, Noshiro, Japan, ²Akita Prefectural University, Akita, Japan, ³Akita Prefecture Forest Technology Center, Akita, Japan.

Asexual propagation, such as layering and sprouting, plays an important role in regeneration system in a natural forest of *Cryptomeria japonica*. Especially, in regions along the Japan Sea, branches are pressed down to the ground by heavy snowfalls every year, and rooted. In order to understand regeneration system of *Cryptomeria japonica* in natural forest, we investigated spatial extension of clones, ratio of sexual and asexual propagations by field investigation and DNA analysis using microsatellite markers.

We established three study plots (P1, P2 and P3) in different forest condition in Akita prefecture, Japan. In plot P1, P2 and P3 all of individuals over 1.3 m in height were investigated for the position in a plot, tree height and diameter of breast height, and needles were sampled for DNA analysis. In each of the plots, core plots (PC1, PC2 and PC3) were established, and all of individuals were analyzed in the same way.

As a result, different regeneration types were detected in each study plot. This result suggested that each study plot is at a different regeneration stage.

P1562. Subterranean development of seedlings into clonal plants

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Subterranean systems of perennials are a complex of organs, their structural components following functional necessities. Our TLP examinations make it possible to observe the underground progress from the seedling to an adult system, enabling us to describe unknown details, such as the transition from a turnip to a secondary fibrous root system in *Potentilla inclinata*.

Growth dynamics follow a three-step progress: (A) establishment of the seedling, (B) survival of an unfavourable season and preservation of site, and (C) cloning in the following seasons, combined with vegetative dispersal.

Furthermore, the development of subterranean systems is a dynamic with two kinetic processes: the vertical movement during seedling establishment brings the innovation buds to a safe soil position, and the horizontal movement during dispersal conquers new sites.

Thus, the "Three steps of progress" provide a fundamental background for acquiring an overall understanding of subterranean plant behaviour.

P1563. Potential costs of division of labor in clonal plants: effects of severing stolon connections between specialized ramets in *Trifolium repens*.M. C. Sampaio^{1,2}, A. Smit-Tiekstra¹, J. F. Stuefer¹;¹Radboud University Nijmegen, Nijmegen, The Netherlands,²Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Connected ramets of clonal plants can morphologically specialize in the uptake of locally abundant resources and exchange local surpluses among them. This phenomenon, termed division of labor, can markedly increase whole-clone performance in undisturbed habitats by enhancing the efficiency of resource extraction from heterogeneous environments. However, division of labor also makes cooperating ramets dependent on each other as they rely on internal resource imported from connected partner ramets. Disrupting stolon connections between specialized ramets should therefore result in potentially costs in terms of ramet mortality and reduced plant growth. We tested this hypothesis in a greenhouse experiment in which we exposed *Trifolium repens* to heterogeneous environments, where the availability of water and light was negatively correlated in space. After specialization we severed the connections between ramets and studied the survival and growth of disconnected clone parts. We found significant costs of specialization in terms of plant performance. We will argue that specialization costs may select against division of labor in disturbed habitats.

P1564. Within-tussock genotypic diversity: Identifying seedling establishment in *Carex sempervirens* tussocks using RAPDsF. Yu¹, J. Schneller², B. Krüsi¹, M. Schütz¹, O. Wildi¹;¹Swiss Federal Institute of Forest, Snow and Landscape Research, Birmensdorf, Switzerland, ²Institute of Systematic Botany, University of Zürich, Zürich, Switzerland.

Carex sempervirens Vill. (evergreen sedge) can form tussocks consisting of densely packed tillers. Previous studies have shown

that seedlings of many other plant species can establish themselves within tussocks of *C. sempervirens*. A question, therefore, rises: Can seedlings of *C. sempervirens* themselves establish also within its tussocks, thereby forming multi-genotypic 'clones'? If they can, is the chance to establish new genotypes (seedlings) higher in weak tussocks than in similar-sized vigorous ones? Comparable number of *C. sempervirens* tillers have been sampled from 12 large, vigorous tussocks (basal diameter [mean \pm 1 SD], 31 \pm 2 cm; tiller cover [mean \pm 1 SD], 24 \pm 6%) and 12 large, weak ones (basal diameter, 30 \pm 2 cm; tiller cover, 88 \pm 9%). Randomly amplified polymorphic DNA (RAPD) markers were used to identify genotypes of the sampled tillers. A preliminary study shows that a *C. sempervirens* tussock can consist of more than one genotype, suggesting that seedlings of *C. sempervirens* may establish themselves within its own tussocks.

P1565. Influence of clonal structure on the selfing rate in *Vaccinium myrtillus* L.

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Clonal growth, which allows the multiplication of flowering shoots of the same genetic individual, can lead to a large floral display and may thus increase the rate of selfing through geitonogamy as a consequence of the increase in the number of flowers visited in succession. The aim of our study is to analyse the combined effect of the diversity and the spatial distribution of clones within *Vaccinium myrtillus* patches on the rate of geitonogamy by a direct estimation of the selfing rate using RAPD markers. Four mother plants were selected within patches characterised by a contrasted clonal structure. The selfing rate was significantly lower for the two mother plants situated within patches characterised by a higher number of clones and a more intermingled distribution of clones. Therefore, in clonal plant species that are not fully self-fertile such as *V. myrtillus*, an increase in the number of clones within a patch and/or an increase in the intermingling of clones could allow a species to have a large multiclonal patch size, and thus a large floral display to attract pollinators, while reducing the effect on the rate of geitonogamy.

P1566. Pteridophytic Flora of Central Himalayas : Rare and Endangered Elements and their Conservation

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On the Indian subcontinent the Himalayas are one of the important centers of plant diversity and richness. The forest area in this region is ever decreasing. Out of the 500 fern species in the Himalayas, this region harbours 350 species. About 10% species of them are endemic. The tree trunk and branches are heavily laden with ferns. Forest destructions have caused huge damage to epiphytic fern vegetation mainly consisting of Polypodioid, Davaloid, Asplenoid, Vittarioid and Hymenophylloid members. Mining and construction of roads in the study area has caused tremendous loss to terrestrial ferns. Common species are becoming rare while the rare species are being completely lost to the Himalayan flora. The list of endangered species of ferns and fern allies in the Himalayas run to about 23. By and large our major concerns today should be to direct our efforts in conservation of members of *Psilotum*, *Huperzia*, *Osmunda*, *Cyathea*, *Botrychium*, *Athyrium*, *Lepisorus*, *Plagiogyria*, *Asplenium*, *Acrophorus*, *Ctenopters*, *Dryopteris*, *Polystichum*, which are in the state of immediate danger of depletion.

P1567. Genetic Biodiversity of *Laurus azorica* (Seub.) Franco in Macaronesia

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Laurus azorica (Lauraceae) is the canopy plant of the Macaronesian laurel forest, an evergreen cloud forest relic of the European and North African Tertiary flora. This shady tree creates optimal conditions for a variety of endemic and endangered island species. It shows different morphotypes in different forest regions (Kunkel 1977) but their genetic background is still unknown. In this study we will analyse the genetic diversity of *L. azorica*, in order to

improve its separation from its sister *L. nobilis* and to reconstruct its immigration, its expansion and its genetic diversity dynamics. So far leaf samples were collected from fragmented forest regions (Tenerife), a larger protected area (La Gomera) and in a natural forest (Azores), and analysed using AFLP markers. Additionally the development of SSR markers is in progress, to investigate population dynamics. These molecular analyses will allow us to look at several questions concerning conservation genetics of *L. azorica*.

P1568. Molecular and biochemical characterization of three different ecotypes of *Oreganum vulgare* grown in Albania.

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Three albanian ecotypes of *Oreganum vulgare* were undergone RAPD analysis and GC analysis. Genomic DNA was isolated from leaves of individual plants according to Gene Elute Plant Miniprep Kit, Sigma. PCR amplifications were carried out according to [Verde, 2002]. The composition of the essential oils was completed via GC. Buds were put in a media (MS, NAA, GA3) while during subculturing and rooting this media (MS) was added BAP : NAA. The ecotypes collected in the areas of Peza, Llogara and Gjirokastra showed genetic polymorphism. The comparison of the constituents in three different vegetative phases showed differences especially in the quantity of thymol and carvacrole. The "in vitro" propagation of the ecotypes showed that the selected media offered optimal conditions for the growth of plantlets; and that ecotypes differed in the rate of development and rooting. Based on the results of the RAPD analysis, essential oils composition, and their attitude toward the "in vitro" propagation conditions we conclude that these ecotypes differ from each other and that they can not be grouped into groups of similarity.

P1569. Influence of human damage actions and soil characteristics in the establishment and development of secondary forest in sand coastal plain vegetation (Restinga Forest) of the littoral area in the state of São Paulo, Brazil

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The sand coastal plain vegetation (Restinga Forest) has been described as an ecosystem associated with the Atlantic Forest, constituted of mosaics. The restinga forest is one of the most fragile and susceptible ecosystems, showing low resilience to human damage. This work was carried out in the Anchieta island, a State Park in the city of Ubatuba in the northern coast of the state of São Paulo, Brazil (45°02' - 45°05' W and 23°31' - 23°34' S). The soil humidity associated with the type and intensity of human damage actions, as well as the natural time regeneration, creating the soil fertility patterns, determined the vegetation physiognomies found in the mosaic of that secondary forest. Based on such information and the plasticity of the most representative plant species, was suggested a model of plantation for natural recovery of gallery forest in Restinga ecosystems.

P1570. Uneven partitioning of genetic variation in chloroplast microsatellites of Swiss stone pine populations along the European mountain range

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Pinus cembra is an important element of European mountain forests. The few regional studies available to date are inconclusive regarding population processes that have led to the current pattern of genetic diversity. We sampled 19 natural populations from the Swiss Alps, representing the central part of the species' natural range, and 9 peripheral populations from the Tatra and Carpathians. Peripheral populations are expected to diverge from central populations as a result of the interwoven effects of isolation, genetic drift, and natural selection. Three chloroplast microsatellites yielded high levels of haplotypic variation in most

populations. In spite of their limited size, the peripheral populations from the Carpathians also displayed high variation, with the most variable population found in the South Carpathians. As expected, the two study regions were genetically separated, and differentiation among populations was lower in the central Alps than in the Carpathians. Therefore the gene pool of the Carpathian populations represents an important component of the genetic resources of *P. cembra* deserving special attention in conservation efforts.

P1571. The relict isolated population of Siberian Spruce (*Picea obovata* Ledeb.) at North-East of Asia.

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The relict isolated population of Siberian Spruce (*Picea obovata* Ledeb.) is situated at the northern coast of the Okhotsk Sea, at the Yama River basin (59°48' and 59°32'N, 153°20' and 153°57'E). This remote refuge is located more than 650 km from the main range of *Picea obovata*, and in fact, is the extreme North-East refuge of this important forest species. This enclave of the Spruce forest is about than 60 km². In spite of long term investigations, it is still a question about precision boundary Spruce distribution.

There is an "island" of the dark-coniferous forests in light-coniferous ("yakutian") and shrub-pine ("beringian") habitats. This refuge serves as a stronghold for a variety of rare and relict plants. Spruce forests have most complex floristic composition and structure among all forest types at the northern coast of the Okhotsk Sea. The spruce patches have a tendency to increase its spread in the Yama valley, however this growth of range is limited by some features of landscape and severe climate conditions which influence the seed ability of trees. It appears, that some individual spruce trees demonstrate growth in conditions far from ecological optimum.

P1572. Modern state of stone exposure's vegetation in the steppe zone of European part of Russia

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Rich experience in investigation of stone steppes and exposures is accumulated in Russia. However, this object concerns with interests of floristic, phytocenology, steppe science, biogeography, phytosociology etc, therefore some necessity is hatched to elaborate the complex of methods for observation of this societies with involving of some modern approaches to the vegetation's classification, geomorphology, mapping and monitoring.

Last years we have comprehensively investigated stone steppes of Eastern Don bass. The great experience is received in vegetation's classification with application of dominant and ecoloco-floristical approaches, the geobotanical maps are elaborated with the GIS technologies of model arrays of stone steppe in its base. Especially for gully systems the original method of geobotanical profiles is elaborated. The great number of endemic and relict species is also revealed.

P1573. Biodiversity of Black Microcolonial Fungi isolated from Antalya/Turkey (Side, Perge, Termessos) and their biodeterioration-potential

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Abstract

In this study rock surfaces of archaeological sites in Antalya were investigated with focus on black fungi for the first time. Black, meristematic fungi were isolated from surfaces of historical marble monuments in Antalya (Side, Perge, Termessos). Their morphology was characterized, their diversity was documented and the taxonomy and phylogeny of new isolates was clarified based on molecular methods, that is, by sequencing parts of the small ribosomal subunit (18 S) and internal transcribed spacer regions. From a total of around 250 samples 111 different fungal strains were isolated. In 72 of 111 strains the rDNA sequencing data and the lack of homologies in "Genbank" gave strong evidence that 72 of 111 strains have to be described as new species and/or genera. All of them, however, clustered within the ascomycete

orders of Dothideales or Chaetothiriales. Field studies show that these organisms cause color changes, black spots, crater shaped lesions as well as chipping and exfoliation of the rock surfaces in Antalya and thus they have to be regarded as a serious threat for the antique cultural heritage in Turkey.

P1574. Phylogenetic relationships in the complex *Galactia-Collaea-Camptosema* (Diocleinae-Phaseoleae-Papilionoideae-Leguminosae) from southern South America

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The genera *Galactia* P. Browne (pantropical), *Camptosema* Hook. & Arn. (neotropical) and *Collaea* DC. (neotropical) are grouped in a complex with exomorphological similarities and different criteria were adopted to delimit them. The phylogenetic hypothesis based on morphological characters do not support the monophyly of *Galactia* and *Camptosema*. Southern South American species were studied including those which generate conflict in the generic delimitation of the complex. The aim of this study was to analyze the phylogenetic relationships of the species by the parsimony method using nuclear (ITS) and chloroplast (trnL-trnF IGS) sequence data. The results obtained did not corroborate the monophyly of *Galactia* and *Camptosema* in agreement with the previous phylogenetic analyses based on morphological characters. The chromosome characteristics of the group were also evaluated and new records for the conflictive species are reported. The results of this study together with the morphological revision point out the necessity of reviewing the generic circumscription of the complex.

P1575. The populations of Seabuckthorn from Zarafshan nature reserve of Uzbekistan as a initial germplasm for breeding

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Seabuckthorn is considered as a valuable fruit and medicinal plant in Uzbekistan.

The area of distribution located mainly in a riparian forests of Zarafshan, Ak-darya and Karadarya rivers. Zarafshan natural reserve is also considered as a principal area of distribution with total area as 64 hectare.

The natural plantations consist of shrubby and tree form with 2,5-10 m of height. The habitus is differed by shape, type and density as well. The fruits are also differed by their color (yellow, yellow-orange, orange, red-orange and red), size (small, medium, large) and shape (round, oval, cylindrical).

The total weight of 100 fruits are from 5 to 20 g. In wild populations it were identified forms with a few thorns and red color. This forms are very interesting for further breeding form. It was determined higher content of fatty oil and carotenoids in this form. The obtained results will help to develop better ways of preservation this plant in the region.

P1576. Mapping quantitative trait loci in multiple populations of *Arabidopsis thaliana* identifies natural allelic variation for trichome density

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The genetic and molecular bases of complex traits are poorly understood. A common approach to this problem is the use of whole-genome scans to identify polygenes, or quantitative trait loci (QTL). The results of such analyses provide estimates of several genetic parameters that may underlie phenotypic variation, including the number of loci, the type and magnitude of their effects, interactions between genes (epistasis), and gene-by-environment interactions. This collection of parameters is often referred to as the "genetic architecture" of a trait. Because these parameters are largely population specific, however, we investigated the potential for variation in genetic architecture by mapping QTL for trichome density in four experimental populations of *Arabidopsis thaliana*. Our mapping results identify nine QTL of significant effect. Through a candidate gene approach, we have cloned one of these QTL. At this locus, we have revealed extensive molecular variation, some of which determines trichome density variation among natural accessions. We shall provide

mapping results, comparative analyses, and interpretations of these findings.

P1577. Genetic Variation in Natural Populations of *Ricinus communis* L : Indirect Ploidy Level Approach

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Abstract

This study aims at finding the cause of the genetic variation observed in natural population of *Ricinus communis* L plant. The hypothesis of different ploidy level was tested using indirect approach. Samples of seeds from populations differing in morphological traits were collected from different localities. The collected seeds were grown in the same experimental field under the same environmental conditions. Two groups of traits were compared. These traits usually used as indirect indicators of differences in ploidy level in flowering plants. The first group included time to germination, plant height, Plant color, time to flowering, flower size, number of fruits per inflorescence, and 100 seeds weight. All these traits have shown significant statistical difference. Other group of traits was stomatal density, stomata size and number of chloroplast in the guard cells.

The results indicate that the cause of the genetic variation in two populations of *Ricinus communis* L is difference in chromosome number. Direct estimation of ploidy level is recommended before initiating any genetic or breeding program using these populations.

P1578. Transformer-3: Automating population genetic analysis.

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The genotyping and the processing of molecular population genetic co-dominant markers need become error-free and much less time consuming in order to implement higher accuracy and urgency in the growing number of applications of this kind of data. In this contribution we introduce Transformer-3, a versatile, user-friendly, proof-reading software that processes drawn interpretations of molecular patterns and genotype or binary matrices introduced by the user to calculate the parameters related to the probabilities of allelic loss and to generate the input files needed to run the programs of most widespread use in population genetics for any possible grouping of populations. The program, still in beta version, is suitable for codominant (allozyme or microsatellite) and dominant (AFLP, RFLP and RAPDs) molecular data for up to 66,000 individuals divided into a maximum of 50 populations. Transformer-3 is programmed in visual basic using a Microsoft Excel® sheet, so it will run in any computer that can contain the Microsoft Office® package. Copies of the current beta version will be made available upon request to the authors.

P1579. Molecular characterization of an Olive (*Olea europaea* L.) cultivar from Abruzzo region

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Olive (*Olea europaea* L., 2n=2x=46), an oil-producing tree, is considered the most important fruit crop throughout the Mediterranean Basin and it is characterized by a large number of varieties, most of which behave in a self-incompatible way. Within a Mediterranean overall cultivation of about 2000 cultivars Italy plays a primary role with its 500 cultivars and, with its 45,248 ha cultivated with olive, the Abruzzo Region is one of the most important areas of olive cultivation in Italy. There are at least 21 cultivars of olive identified in Abruzzo. Within these, Dritta is one of the most popular cultivar because of its medium-high yield in oil (18-20%) that is of high organoleptic quality. Since the characteristics of an olive oil, as well as of other crop-derived products, depends both on the area of cultivation and genetic constitution of cultivars, a germplasm characterization represent a key factor for the traceability of the final product. Here we report molecular characterization of 21 accessions of DRITTA cultivar compared with 8 of the most cultivated varieties in the Abruzzo region.

P1580. Multiple evolution of monoecy from dioecy in *Momordica* (Cucurbitaceae)

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The African/Asian genus *Momordica* comprises 47 species, all with unisexual flowers. Phylogenetic analyses of chloroplast gene, intron, and spacer sequences from all species show that *Momordica* is monophyletic and sister to the Asian *Siraitia*, *Indofevillea*, *Baijania*, and *Thladiantha*, all dioecious. The eight Asian *Momordica* species also are dioecious. Of the 39 African species, 20 are dioecious and 19 monoecious, and monoecy apparently evolved five times. Pollinator discrimination against female individuals may be among the selective factors favoring monoecy: With the exception of the widely cultivated *M. balsamina* and *M. charantia*, *Momordica* species are pollinated by *Ctenoplectra* bees, which obtain pollen, nectar, and fatty oils from the male flowers. Female flowers are similar in morphology, but almost or entirely rewardless. Where *Momordica* occurs at low densities, such as in savannas, pollinators may avoid females, favoring cosexuality. Species of humid forests are mostly dioecious. Fieldwork will test this hypothesis.

P1581. Assessment of genetic variation of common juniper (*Juniperus communis* L.) in Luxembourg

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In order to assess the conservation value of small stands of common juniper (*Juniperus communis* L.), we studied the genetic population structure of this rare shrub in Luxembourg. In 10 populations spread throughout the range, we sampled up to fifteen plants along transects. To test for genetic structuring within the populations, more than one transect was used in the largest populations.

Most genetic variance was found within populations (81% of total variance), although there was a significant genetic differentiation (RAPD patterns) between populations (10%) ($P < 0.05$) and among transects within populations (9%) ($P < 0.002$). The mean number of individuals exchanged per generation between transects ($N_{em} = 1.06$) and the similar amount of variance within and between populations indicate a moderate gene flow within the study region. This could be explained by wind pollination and seed dispersal by birds. It might also reflect a wider distribution of this long-lived plant in the recent past before changes in agricultural practices led to increased fragmentation of its habitat.

P1582. High levels of genetic diversity in *Dioon caputoi* De Luca, Sabato and Vázquez Torres (Zamiaceae, Cycadales) a narrowly distributed species of Puebla, México.

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The amount and distribution of genetic diversity within and among four populations of *Dioon caputoi*, a narrowly distributed cycad of Puebla state in México, was determined. High levels of genetic variability was found ($P = 78.95\%$; $A = 1.91$; $H_e = 0.350$), compared with other species of cycad in the Zamiaceae. In spite of the small population sizes (between 50 and 120 individuals) there was no significant global nor local inbreeding ($F_{it} = -0.242 \pm 0.09$; $F_{is} = -0.379 \pm 0.08$, respectively). Rather, a significant excess of observed heterocigosity with respect to Hardy-Weinberg equilibrium expectations was obtained in the four populations studied. However, since the analysis of bottleneck showed a significant deviation from the mutation-genetic drift equilibrium model in the four populations (that is, a transient excess of heterocigosity) this high level of genetic diversity could be explained as a recent reduction in population sizes. Biogeographical and ecological implications are discussed.

P1583. Allozyme diversity of diploid and dysploid species of *Delphinium* series *Fissa* (Ranunculaceae) in the W Mediterranean area

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The genetic diversity and population structure of *Delphinium* series *Fissa* taxa (Ranunculaceae) in the Western Mediterranean area is assessed using allozyme electrophoresis. This group comprises four taxa (*D. bolosii*, *D. mansanetianum*, *D. fissum* subsp. *fissum* and *D. fissum* subsp. *sordidum*), most of them narrow endemic and endangered plants. Dysploid ($2n=18$) and diploid ($2n=16$) species have been reported for this series. Within the 12 surveyed populations, a total of 15 loci were interpreted and 30 alleles were identified. Low levels of genetic diversity were found for all taxa, the most diverse was the dysploid *D. bolosii* ($P=28.9\%$, $A=1.4$ and $H_e=0.104$) and the less diverse *D. mansanetianum* ($P=6.7\%$, $A=1.2$ and $H_e=0.071$). High levels of divergence were estimated for *D. fissum* subsp. *sordidum* populations, while a taxa-specific allele (*Idh-1b*) was found for *D. fissum* subsp. *fissum*. Nei's genetic identity index was also calculated suggesting a close relationship between the recently described *D. mansanetianum* and *D. bolosii*. Given their threatened status, these data are helpful for management strategies and conservation plans for these taxa.

P1584. Simple sequence repeat (SSR) analysis for assessment of genetic variability in apricot cultivars

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The genetic diversity of apricot (*Prunus armeniaca*, $2n = 16$) was studied using SSR markers. A total of 129 apricot cultivars from different geographic origins of Corvinus University (Budapest, Hungary) and BOKU (Vienna, Austria) were selected. Thirty microsatellites primer pairs, developed in apricot were used. Ten of them amplified polymorphic patterns, which was useful to investigate patterns of variation in apricot cultivars.

The UPGMA dendrogram based on the genetic distance reflected the grouping of cultivars in agreement with their geographic origin and pedigree. Eastern European cultivars (Hungarian) belong to four subgroups, which share the same parentals in their pedigrees. Three subgroups of the Hungarian cultivars seem to be more closely related to the Western European than to the Central Asian cultivars, while one subgroup more close to the Central Asian group.

Asian and American cultivars were intermediate showing a different genetic basis than the Eastern European cultivars.

The implications of these data for the use of SSR fingerprints in breeder's rights protection and apricot breeding are discussed.

P1585. RAPD variation of the regional endemic *Saxifraga rosacea* subsp. *sponhemica* in Europe

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We studied the genetic population structure of the rare rock plant *Saxifraga rosacea* ssp. *sponhemica* which occurs in naturally isolated populations in several European countries. In 34 populations we sampled up to fourteen plants each along transects. To test for genetic structuring within the populations, more than one transect was used in four large populations. There was strong genetic differentiation (RAPD patterns) among countries (15% of total variation), among populations within countries (21%) and between transects within populations (11%) (all $P < 0.001$). The strong genetic variation among populations and the mean number of individuals exchanged per generation between transects ($Nem = 0.27$) indicates that gene flow is low. The significant genetic differentiation between transects within populations indicates little gene flow even within populations. Possible explanations for the generally low gene flow are pollinator behaviour and restricted dispersal of the seeds. Genetic differentiation among all 38 transects (pairwise Φ_{ST}) was related to geographic distance (Mantel test, $r=0.539$, $P<0.0001$).

P1586. Genetic variability and fertility of *Vriesea gigantea* (Bromeliaceae) populations from the Rainforest in South Brazil

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Vriesea gigantea Gaud. is a bromeliad species occurring in the Atlantic Rainforest, eastern Brazil. Little is known about the genetic variability, fertility and viability of bromeliads populations. The aims of this study are: to describe the genetic variability by using SSR

molecular markers in populations of *V. gigantea*, and to analyze the fertility and viability of these populations throughout meiotic behaviour in pollen-mother-cells, pollen viability, and seeds germination. Five SSR primers developed by Boneh et al. (2003) for *Tillandsia* and *Guzmania* genus will be used. The primers were tested on 12 individuals from different locations in the south Brazil. Three primers have amplified positively (EB6 -3alleles; E6 -2alleles; and P2P19 -8alleles) under the same PCR conditions described by the authors. The heterozygosity observed to each primer were: 0.125, 0.8, and 0.875, respectively. Optimized PCR conditions are being developed to the others two pair primers (CT5 and E19). These results are promising for the utilization of these markers to study genetic variability of related species of *Tillandsia* and *Guzmania* genus such as *V. gigantea*.

P1587. Genetic investigation of African willows along the Blue and the main Nile rivers in the confluent region of Khartoum (Sudan)

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The recent increase of human activity along the Nile deeply affects this floodplain ecosystem. Willows represent a reliable solution to the decontamination of water as well as the stabilisation of the riverbanks. However, till now little is known about the genetics of both native *Salix subserata* Willd. and *S. muriei* Skan. The ploidy level of both species was determined using flow cytometry. At the chloroplast level, one haplotype of each species was identified by sequencing of five regions of the chloroplast, PCR-RFLP and SSCP. The nuclear genome was analysed using microsatellite primers originally developed for *Salix burjatica* and *Populus* sp. The results show the presence of vegetative reproduction, as 13 % were clones. Multivariate analysis (PCA) of the genetic data reveals hybridisation and introgression of both species in the studied areas. Results of microsatellites analysis show a low genetic differentiation among the investigated populations (F_{ST} value = 0.13). This low genetic diversity might be attributed to the high gene flow between populations, through pollen flow and/or seed dispersal rather than to the quite low vegetative reproduction.

P1588. Molecular evidences for repeated hybridization events as the origin of the genus \times *Crepidiastrixeris* (Asteraceae) using RAPDs and ITS data

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The hybrid origin of \times *Crepidiastrixeris denticulato-platyphylla* was investigated using RAPDs and ITS sequence data. The putative parents *Paraixeris denticulata* and *Crepidiastrum platyphyllum* represent separate species, irrespective of geographic origin. The combined occurrence of species-specific RAPD markers from *P. denticulata* and *C. platyphyllum* in \times *C. denticulato-platyphylla* established unambiguously a hybrid origin between the two taxa. This is in line with the occurrence of a combination of morphological characters, such as plant habit and floret numbers. The parent taxa differed by 7 nucleotide substitutions and two indel events from each other in the ITS region. The hybrids showed sequence additively and mostly represented F_1 plants, with the exception of two plants which were of putative F_2 origin. The present study suggests that \times *C. denticulato-platyphylla* exists due to frequent hybridization between the two species of *P. denticulata* and *C. platyphyllum*.

P1589. Genetic diversity studies on *Mallotus philippinensis* Muell. (-A Dye yielding plant) using Isozyme data

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Isozyme analysis has been applied widely to characterize the patterns of genetic differentiation in plant species. Genetic characterization of natural resources is an essential step for a better understanding of genetic resources for the implementation of in situ and ex situ conservation activities. So adequate

knowledge about the plant is necessary for planning sustainable development of any region like India, where the flora is rich in diversity and endemism. *Mallotus philippinensis*, belongs to the family Euphorbiaceae, yielding red colour dye from its fruit was used in ancient India, by the Hindu silk dyer. Genetic diversity was assessed on seeds collected from 10 different locations and twelve different enzyme systems were screened and forty two putative loci were totally resolved with sufficient consistency and clarity. Cluster analysis showing the great genetic variations among the population. The outcome of this study, it is hoped, would help to exploit populations that would show the greatest genetic diversity for improving the dye content through future studies. Also these results have important implications for the conservation strategy.

P1590. Genetic structure of an ectomycorrhizal fungus *Chroogomphus rutilus* population in a *Pinus tabulaeformis* forest

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The genetic structure of an ectomycorrhizal fungus *Chroogomphus rutilus* population was evaluated in a *Pinus tabulaeformis* stand at Dongling Mountain of north China using random amplified microsatellite (RAMS) markers. Two hundred and ten sporocarps were collected at two stands and divided into 108 genets analyzed by three 5'-anchored RAMS primers. Among these genets, 75 (69.4%) were represented by single sporocarps, the other 33 comprised multiple sporocarps. The genetic diversity as determined by Shannon-Weaver diversity index was 2.92 in this population, furthermore, there were similar diversity indices within stand A (2.89) and stand B (2.92). The largest genet is up to 450 m. The genets within a similar group located close to each other. The results indicate that *C. rutilus* population in mature forest stands can comprise large genets or small genets, and suggest that it is very important to spread *via* below-ground mycelia, as well as sexual spore propagation in the life history of the *C. rutilus* population.

P1591. Morphometric circumscription of species and infraspecific taxa in Tunisian *Pseudophrys* (*Ophrys*, Orchidaceae)

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The section *Pseudophrys* is a taxonomically critical species complex with pronounced morphological variation, suggesting the need for further taxonomic evaluation, specially in Mediterranean Africa, a centre of radiation for orchids of this section of the genus *Ophrys*. The taxonomy of *Pseudophrys* in Tunisia has been controversial. A multivariate analysis has been performed in order to study the polymorphisms of the twelve species represented in Tunisia. Extensive observations in the field and analysis of morphological characters show that most of the characters used by previous authors for distinguishing the species are variable between or even within populations. To determine characters distinguishing different species and to examine their circumscription, we performed morphometric analyses on three different subsets, one with all the currently recognized species, other two containing the closely related *Ophrys eleonora*/*O. vallesiana*, and *O. africana*/*O. gazella*. These populations were treated as Operational Taxonomic Units (OTUs) and studied using cluster analysis, principal component analysis and canonical discriminant analysis.

P1592. Genetic diversity of Kentucky bluegrass (*Poa pratensis* L.) populations evaluated by RAPD markers

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Random amplified polymorphic DNA (RAPD) markers were used to measure genetic diversity in germplasm collection of Kentucky bluegrass (*Poa pratensis* L.). Total of 20 populations (local varieties and cultivars) were collected from Slovakia, Czech Republic and Ukraine. From 12 tested primers, a 23 bands (in average 12 bands per population) was detected by two 10-mer RAPD primers PP2 5'CCGcAgTCTg3' and PP3 5'ACATcGCCCA3'. Nei and Li's index of the genetic distance was used to class diversity between populations. This index showed low genetic diversity, in average was 0,288, but second primer PP3 detected higher genetic

diversity. Cluster analysis UPGMA was used to illustrate the diversity of genetic material. This analysis detected 4 populations (South Ukraine, 63/718, 63/721 and Maly Slizovnik) with high genetic diversity. They created different cluster of all populations. Optimized PCR RAPD reaction and RAPD primers PP2 and PP3 could be used to detect genetic diversity between populations and could be used in plant breeding program of Kentucky bluegrass.

P1593. Isozyme variation in natural populations of *Pterocarpus macrocarpus* Kurz. in Vietnam

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ABSTRACT

We analysed genetic variation within and between populations of *Pterocarpus macrocarpus* from Vietnam to improve conservation of genetic resources of this hardwood species. A total of 651 seedlings from 61 trees and from three locations were tested at 10 polymorphic loci. Of 11 isoenzymes investigated, malate dehydrogenase (MDH), peroxidase (POD) and esterase (EST) were polymorphic at all 10 loci. Percentage of polymorphic loci per population ranged from 54% to 64.5% with a mean of 60.5%. Within each family, the frequency of isoenzyme phenotypes ranged from 0 to 1 with a mean of 0.5276 per locus. An UPGMA tree was constructed based on the Euclidean distance matrix and isoenzyme variation. This showed no association with geographical distance but evidence for isolation along longitudinal gradient. A Neighbor Joining (NJ) tree was constructed based on isoenzyme phenotypes and this showed one family (Nghe An) was dispersed from other two families (Tay Ninh and Yokdon). An ordination plot of the principal coordinate analysis confirmed these results.

P1594. Spatial differentiation and estimation of immigration rates with a stepping-stone structure for *Fagus crenata* populations in Japan

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Spatial genetic substructurings of a local *Fagus crenata* (Siebold's beech) population in central Honshu (Japan) was analyzed by 13 allozyme loci. This beech forest has been rapidly deteriorating by air pollution and feeding pressure of sika deers. Spatial autocorrelation analysis revealed positive significant values within small distance classes and negative in farther classes. Twelve patch populations were discriminated within the local population, and a simulation analysis was conducted using the stepping-stone model to estimate the migration rate among patch populations. The population migration rate ($Nm=2.7$) was revealed to be larger than one migrant every other generation, which indicates that the population differentiation is not significant. Although the model analysis demonstrated a sufficient gene flow between neighboring patch populations, the severe damage to local populations may cause the fragmentation of patch populations.

P1595. Morphological variation of *Spiraea* (Rosaceae) from Siberia and the Far East (Russia).

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About 17 kinds of *Spiraea* grows in territory of Siberia and the Far East Russia. Various forms of variability of morphological attributes of disputable kinds are investigated. Variability within the limits of one plant of metric attributes of the kinds concerning to miscellaneous sections of *Spiraea*, is characterized by close parameters that testifies to specificity of attributes and presence of the general laws of variability. The analysis of variability of metric attributes between plants of one population and between populations specifies specificity of kinds. The majority of metric attributes (the length of a sheet, width of a sheet, length of kidneys etc.) the investigated kinds are characterized by the expressed variability in east and southeast directions. Geographical law in variability of qualitative attributes (color of axes of inflorescences etc.) it is not revealed.

P1596. Vegetation survey of the northwest part of the Šar-Planina Mt.

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Šar-Planina Mt. belongs to the Central Balkans' massif and is 85 km long. The main ridge is about 2,500 m high. The southern slopes of the massif belong to Macedonia, the northern and northwest ones to Serbia, while the smaller part of the southwest slopes to Albania. Specific geographic location, where the Mediterranean and continental influences clash and imbue, the great relative height difference, the variety of geological base, outstanding soil plasticity with the dominating glacial morphology, rich floristic composition, as well as entanglement of different ecological conditions constantly changing in a small area, have produced an extreme vegetation variousness of the region. By long-range terrain research of the Šar-Planina Mt., as well as revision and grouping of all so far obtained plot data, 222 vegetation units, i.e. 136 plant associations classified into 47 alliances, 23 orders and 16 classes have been found. In the paper shall be given their syntaxonomic survey.

P1596a. Large scale AFLP screening of natural populations provides the basis for the analysis of the molecular mechanisms underlying biodiversity - the case of *Melampyrum* (Orobanchaceae)

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Analyses of mutants and "ecotypes" using mainly seedbank material cover only a small part of the variation present in natural populations. By contrast, in nature there is a large range of variation, encompassing an unknown number of distinct ecotypes, varieties and mutants.

Within the 6 species of *Melampyrum* known in Trentino/Italy, AFLP analyses revealed considerable genetic distances within and between populations as well as differences between species concerning population- and meta-population structure. Beside several morphological and ecological traits varying between populations and species also a flower-mutant independently appearing in different populations was discovered. Furthermore there is a lack of correlation between morphological and genetic distances between the species to the point that even a 7th species was found.

While genomic-based AFLP provides the basis to define the populations or species to be compared, other techniques, like cDNA-AFLP are used to identify the genes involved in the traits analyzed.

A summary of our approach to trace the molecular mechanisms underlying biodiversity by analysing natural populations is given.

P1597. Genetic diversity of an invasive plant *Eupatorium adenophorum* analyzed by AFLP marker

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Crofton weed (*Eupatorium adenophorum*) is a damaging invasive weed that has been established in more than 30 tropical and subtropical countries worldwide. AFLP technique was used to analyze genetic diversity of 24 populations of Crofton weed collected from 24 locations in China and Australia. AFLP analysis produced 509 scorable bands, of which 77.01% were polymorphic. The related diversity indexes: na was 1.50, H 0.29 and I 0.42, indicating that genetic diversity is very rich. The cluster analysis showed that in Yunnan Province first invaded in China, it had great genetic variability among geographically close or far populations because all ones sampled in this province could be classified into eight groups. In other newly-invaded areas, genetic diversity was simple and distinctly related to the geographically close populations collected from the place of Yunnan, which spreading seeds by wind resulted in. Moreover, water can carry the seeds to far regions from the seed-produced place.

P1598. After the bottleneck: patterns of introduction and rapid evolutionary change in an invasive grass

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Barbed goatgrass, *Aegilops triuncialis*, closely related to cultivated wheat, is a cleistogamous allopolyploid annual grass with a native range from the Mediterranean to Afghanistan. *Ae. triuncialis* was first introduced into California in 1914 and is now considered to be a serious noxious range weed. It is found more commonly on infertile soils, such as serpentine outcrops, than in richer zonal soils. This makes goatgrass a threat to fragile habitat types.

Using nuclear and chloroplast loci we examined the history of invasion of *Ae. triuncialis* into California. Analysis of 170 accessions from California and Eurasia indicates that a strong bottleneck occurred during its introduction.

Of 38 species wide genotypes, 2 are present in California, and these 2

appear to separate into two broad groups. This indicates that there were two separate introductions.

The ability of *Ae. triuncialis* to invade serpentine soils, a habitat that is not occupied by goatgrass in its native range, suggests the possibility that some lineages adapted to new habitats following range expansion. Ecological and molecular studies to answer this question are currently underway.

P1599. Does allelopathy explain the invasion success of *Solidago canadensis*?

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The success of plant invaders has been tried to explain by the loss of natural enemies in the newly invaded range leading to compensatory release and enabling the species to allocate their energy into growth and reproduction instead of defence. However, in *S. canadensis* s.l. L. such a compensatory release has not been found in a comparative study. A further mechanism explaining the success of invasive weeds may be the production of chemical, allelopathic compounds by the invader that have harmful effects on plant neighbours that have not been co-evolved. We took root extracts of *S. canadensis* and performed thin layer chromatography to recognize the compounds. The compounds were individually tested on filter paper on their putative negative impacts on germination of seeds of competing plant species in the invasive range of *S. canadensis*. In addition, in a pot experiment, competing plant species were grown with *S. canadensis* either with activated carbon in the soil adsorbing the allelopathic compounds or without to check whether allelopathic effects of *S. canadensis* might lead to a competitive advantage

P1600. Evolution of increased competitive ability

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The invasion success of introduced species depends on the interaction of habitat invasibility and species' invasiveness. There is increasing evidence that not only the intrinsic plant characteristics, but also post-introduction evolutionary changes are essential. They require a high additive genetic variance, which appears to be common among traits facilitating invasion. Adaptive evolutionary changes have been demonstrated for a number of plant species, although the driving forces or selective factors appear to differ in different environments.

Among other selection pressures, the absence of specific herbivores in the introduced range can be fundamental and, as postulated in the Evolution of increased competitive ability (EICA)-hypothesis, may lead to an altered trade-off between herbivore defence and competitive ability. Generalist herbivores may have the opposite effect, causing a selection for better-defended genotypes rather than highly competitive ones. Both theories seem to apply for some species and under certain environmental conditions.

P1601. Microsatellite isolation in native (*C. prolifera*) and invasive (*C. taxifolia*) *Caulerpa* sp in the Mediterranean sea

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Caulerpales are clonal marine algae, which often act as invasive species. Over the past decade, *C. taxifolia* has spread in along the Mediterranean coast, presently occurring at 70 sites and covering nearly 3000 hectares of subtidal area. About 60 km of coastline are currently affected and there is concern about the potential reduction in biodiversity and changes in coastal ecosystem

functions. Information about population mechanisms of population maintenance and spreading in both species are largely lacking in order to assess clonal structure and genetic diversity of recently established populations of the invasive species *C. taxifolia* in comparison with populations of the native *C. prolifera* in the Mediterranean, new genetic markers (microsatellites) are being developed. Two genomic libraries enriched for microsatellite dinucleotide repeats have been constructed per for each *Caulerpa* sp. Several microsatellite sequences have been obtained and the levels of polymorphism within those sequences are being tested.

P1602. Genetic differentiation between native and introduced populations of the South African ragwort *Senecio inaequidens*

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Invasive species often evolve rapidly in response to the novel biotic and abiotic conditions in their introduced range. Such evolutionary changes might play an important role in the success of some invasive species. Here, we investigated whether introduced European populations of the South African ragwort *Senecio inaequidens* have genetically diverged from native populations. We carried out a common garden experiment where 12 South African and 11 European populations were grown at two levels of nutrient availability, as well as in the presence or absence of a generalist herbivore. We found that, in contrast to a current hypothesis, plants from introduced populations had a significantly lower fitness, and they were more resistant to herbivory. Moreover, introduced populations were less genetically variable, but displayed greater plasticity in response to fertilization. We argue that in order to understand the invasion success of *Senecio inaequidens*, its pathways of introduction must be taken into account. Overall, our data support a model in which both adaptive evolution and phenotypic plasticity contribute to invasion success.

P1603. Increased susceptibility to enemies following introduction in the invasive plant *Silene latifolia*.

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One of the influential hypotheses why species become invasive in their introduced ranges is that release from natural enemies allows them to shift investment from traits involved in defense to traits enhancing growth, reproduction and competitive ability. The invasive plant *Silene latifolia* was introduced from Europe (EU) into North America (NA) approximately 200 years ago and experiences lower damage by natural enemies in the introduced range. A common garden experiment in Europe, using seeds from 20 EU and 20 NA populations reveals (1) genetically-based differences in life history between plants from EU and NA populations; plants from NA have evolved a weedy, invasive phenotype that flowers two weeks earlier and has a two- to three-fold higher reproductive potential; (2) higher susceptibility of plants from NA populations to fungal infection, fruit predation, and aphid infestation, suggesting that the invasive NA phenotype has evolved at the expense of the loss of defensive abilities.

P1604. Molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America

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Invasive species offer excellent model systems for studying rapid evolutionary change. Molecular markers can provide information about pathways of introduction, the amount of genetic variation introduced and the extent to which founder effects and inbreeding after bottlenecks may have contributed to evolutionary change. We studied microsatellite variation among and within 27 native and 26 introduced populations of garlic mustard (*Alliaria petiolata*), a European herb invasive in North America. Overall, introduced populations were genetically less diverse. However, considerable variation was present and when compared to the likely source regions no bottleneck was evident. The high allelic diversity in the introduced range strongly suggests multiple introductions. Out of six European regions, the British Isles, Northern Europe and Central Europe had significantly higher proportions of alleles in

common with the introduced range, and are therefore the most likely source regions. The analyses will be updated by the results of ongoing investigations using chloroplast DNA markers from which we hope to get more insight into the pathways of introduction.

P1605. Genetic variation among native and invasive populations of *Senecio jacobaea* and its correlation with invasive traits

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Senecio jacobaea is native in Europe and has become a noxious weed in North America, Australia, and New Zealand. In a comparative study using AFLP data, genetic diversity among invasive populations from North America, Australia and New Zealand was substantially smaller than among native populations. Invasive populations all belonged to the same chemotype whereas the genetically more diverse native populations could be separated into two distinct chemotypes. Invasive populations had a higher performance, i.e. showed higher vegetative and reproductive growth in comparative experiments and also produced more pyrrolizidine alkaloids, their main antiherbivory compound. Compared with natives that have to deal with an array of both specialist and generalist enemies, invasive populations benefited from high protection against a generalist herbivore, but lost additional protection against chemically adapted specialist herbivores absent in the invaded areas.

P1606. Similarity in the adaptation of life-history traits to latitude and genetic differentiation between native and introduced populations of *Mimulus guttatus*

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Latitudinal similarity and post-introduction evolution may be important for plant invasions. In a common environment, we compared life-history traits of plants of *Mimulus guttatus* from its non-native ranges in New Zealand and Scotland with plants from the whole latitudinal gradient in its native range in North America. While phenology was not affected by the latitude of origin, plants from higher latitude produced fewer flowers but more and longer stolons. These latitudinal clines did not differ between the native and non-native plants, indicating that non-native plants have the same reproductive allocation as native plants from similar latitude. Plants from the non-native ranges, however, had larger flowers and produced more branches than the ones from the native range, indicating that they have evolved differently. Moreover, populations from the non-native range had lower heritable genetic variation for time to anthesis and anther-stigma separation than the ones from the native range, indicating a lower potential for their evolution in the non-native ranges. We conclude that evolutionary processes play an important role in the invasion by *M. guttatus*.

P1607. Cultigenes as a source for neophyta? - Feral oilseed rape and its putative natural hybrids in northern Germany

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Oilseed rape is well-known for its innate characteristic of establishing feral populations outside cultivated areas. Furthermore, hybridisation between *B. napus* and its close relatives such as *B. rapa*, *B. oleraceae* and *Raphanus spec.* under field conditions has been well documented. Populations of feral oilseed rape, close relatives and potential hybrid populations in Osnabrück and its surroundings are recorded in this study. Out of 77 populations sampled in the area under study during the first vegetation period, two populations have been identified as potential hybrids by morphological and cytological (chromosome number, flow cytometry) analyses. Populations are investigated by flow cytometry in order to identify hybrids of different ploidy levels from various provenances. In addition molecular markers are also applied to compare inter- and intra-population variability of oilseed rape and to analyse relationships between feral populations, putative hybrids and cultivated varieties. To check the persistence of feral oilseed rape populations the area is going to be monitored during several vegetation periods.

P1608. Comparison of native and invasive populations of *Senecio inaequidens* for vegetative and reproductive traits in a control common garden experiment

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Senecio inaequidens, a South African species, has accidentally been introduced into several sites in Europe where it has become an invasive. Its range now extends from the Mediterranean coast to Denmark.

Only tetraploid individuals are found in Europe whereas diploids and tetraploids are present in Africa. We compared vegetative and reproductive traits of individuals sampled as seeds in two tetraploid populations from South Africa, two populations from France and two populations from the Netherlands, these two latter countries representing two different introductions.

Seedlings were planted outdoors in Montpellier France into three different environments: control (Mediterranean-type), heated (night screens and heating cables) and water supplied (Dutch mean precipitations).

Mean character values did not show differences between Africa and Europe. Several significant interactions between environments and countries were found, showing that plasticity for those characters varies with the country of origin. These experimental data will be used to develop a process-based biogeography model to predict the spread of *Senecio inaequidens* in Europe.

P1609. Polymorphism in seed of *Acer pseudoplatanus* L.: differences in responses to phytohormones and stratification

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Seed mass, as most relevant measure of seed size occupies important position in the ecology of a species. It links the ecology of reproduction and seedling establishment with the ecology of vegetative growth (Leishman & Westoby, 1992). Maternal factors can markedly influence seed dormancy breaking and germination requirements. Production of two or more different types of seeds by a species (seed polymorphism) is found in a number of species (Gutterman, 2000). This phenomenon regulates temporal distribution of seed germination.

Strong invasive properties made sycamore one of the most common tree species in urban areas of Europe. In this species were identified two general type of inflorescence (protogynous and protandrous inflorescence). Morphological differences and fruit size variations in infructescens, between protogynous and protandrous individuals were reported, (Binggeli, 1990).

The results show differences in responses to phytohormones and stratification of two seed types. It was found that stored GA significantly influences germination of large seeds in contrast to small, where *de novo* synthesis of GA is necessary to complete germination.

P1610. Dispersal pattern of asexual and sexual propagules in bryophytes

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Dispersal ability is of great importance for plants, which commonly occupy spatially limited habitat patches. Bryophyte dispersal has been thought to take place primarily by spores. Another potential mechanism is dispersal by asexual diaspores and leaf fragments. In this presentation, we report results concerning the dispersal of spores and gemmae in the leafy hepatic *Anastrophyllum hellerianum* in Southern Finland. *A. hellerianum* is a dioicous epixylic hepatic which reproduces both sexually by spores and asexually by one-celled gemmae. Lower reproduction investment is required for asexual than sexual reproduction in *A. hellerianum*. As the production of spores is rare, the ability of species to colonize new substrate patches by frequently produced gemmae is crucial. The study was conducted both in a natural habitat and in an artificial set-up without background dispersal. Traps were distributed in different directions at distances of 0-10 m from the parent colony. The results indicate that asexual gemmae may function in dispersal over relatively long distances, which is comparable to the dispersal of spores on a local scale.

P1611. A seed pool approach to tracking acorn movement by acorn woodpeckers

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Animals are the principle vectors of seed dispersal for many plant species. Unfortunately, tracking animals and dispersed seeds is a long-standing challenge. We introduce a novel approach that uses maternally-inherited seed tissue to quantify the genetic structure of dispersed seed pools. This approach measures the genetic variability within and among seed pools, without requiring a highly polymorphic battery of markers, or the locations and genotypes of all possible seed parents. We demonstrate this approach with the specific case of seed transport of valley oak (*Quercus lobata*) acorns by acorn woodpeckers (*Melanerpes formicivorus*). These territorial birds store acorns in drilled holes in the bark of trees called granaries. An analysis of 3 microsatellites derived from maternally-inherited DNA in seed coats from acorns stored in different granaries shows extremely high genetic structure in the seed pool among granaries, a low number of effective seed donors per granary, and restricted seed movement. Our findings provide insight into the foraging and seed-dispersal behavior of acorn woodpeckers, with an approach that can be widely extended to other systems.

P1612. Successful colonization and survival of tropical macrophyte *Pistia stratiotes* in thermal waters of temperate zone

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Temporary occurrence of *Pistia stratiotes* outside the tropical or subtropical zone is not rare, but winter survival outdoors was not reported so far. Few plants were spotted on the thermal waters near Catež (Slovenia, 45°53'N 15°37'E) in 2001 for the first time. The species might have been introduced by incidental release of specimens used as ornamental pond plant. In 2002 it spread out and formed dense mats, which in 2003 completely covered the 25ha surface of two natural thermal water bodies with temperature values between 25°C and 31°C whole year round. A gradient of declining nutrients was perceived and correlated with leaf traits, plant biomass, plant density and flowering. Extremely large plant size (rosettes diameter up to 64cm) was observed, probably due to favourable nutrient status, temperature conditions and longer photoperiod as within its natural range. Plants bloom and produce viable seeds, which were already present in the water sediment in 2004. The seasonal changes in biomass follow the local climate, having the peak in summer and strong decline in winter, when most of leaves decay, but many small rosettes survive and resprout quickly in early spring.

P1613. Sex Allocation Bias in Hermaphrodite Plants: Effects of Local Resource Competition and Seed Dormancy.

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We developed a general model that describes the relationships between sex allocation and spatial/time dispersal of plants. Sex allocation tends to be male biased in animals when daughters don't quite disperse than sons because they must compete with daughters or between mother and daughters for locally limited resources (local resource competition [LRC]; Clark, 1978). We applied LRC effect to hermaphrodite plants and developed a game model to examine whether local resource competition with relative seeds effect the ESS sex allocation. We found that e.g. when selfing rate is relatively low and pollen are relatively easy to disperse out of their patches than seeds, ESS sex allocation becomes more male biased. In addition, most plants' seeds are known to have partial dormancy. Dormancy is understood as not only a bet-hedging strategy for an unpredictable environment, but also offspring dispersal strategy over time axis. To develop more general and practical model, we also analyzed whether ESS sex allocation changes when considering a certain seed dormancy rate further.

P1614. Gene flow, pollen competition, and introgression among cultivated and wild populations of *Lobelia cardinalis*

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To understand risks to local adaptation due to influx of genes from non-local populations of *Lobelia cardinalis*, I evaluated the potential for gene dispersal from small plantings into wild populations, probability of hybridization and consequences of introgression. The likelihood of gene movement from ornamental plants to wild populations is high, and chances of establishment of hybrids in natural populations are good.

Pollinators did not discriminate between local and non-local pollen, fertilizing half the flowers 1 km from a pollen source. Testing siring ability of non-local pollen donor populations showed that pollen source affected the percentage of locally-sired offspring, but direction of bias was not consistent among donors. A field experiment with hybrids of local and non-local populations revealed a high likelihood of hybrid establishment. While local parentals out-survived other parentals, F₁ hybrids survived even better. Hybrid generations reproduced in the field, allowing introgression of non-local traits. A parallel study in controlled conditions showed little genetic differentiation among parentals and among resultant hybrids.

P1615. Natural hybridization in mangroves: molecular approach to hybrid identification, maternity confirmation and introgression

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Interspecific natural hybridization is important in plant evolution. The occurrence of natural hybridization is not random, and varies in frequency among families and genera. Natural hybrids are only known in three genera of major mangrove families, recognized based on morphological criteria. Many morphological traits are subject to environmental influences, and to complications arising from character convergence and homoplasy, so neutral molecular markers should be used to verify natural hybridization, introgression and maternity. Natural hybridization in three major mangrove genera, *Bruguiera*, *Rhizophora*, and *Sonneratia*, was examined using ISSR, cpDNA and ITS sequences. All natural hybrids in these genera, resulting from interspecific hybridization between closely related taxa, were confirmed. The maternal species involved in the hybridization were identified. The suitability of ISSR markers was shown for detecting introgression in *Bruguiera* populations that occupy a wide geographical area. The evolutionary consequences of potential gene flow among sympatric species through introgressive hybridization are discussed.

P1616. A microsatellite analysis of the genus *Micromeria* Benth. (Lamiaceae) in Macaronesia

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Macaronesia shows high plant species diversity. Adaptive radiation is assumed as one reason for the high amount of endemic taxa for several plant groups. One example is the genus *Micromeria* with 16 species recognised in Macaronesia. Most are narrow endemics, only few are distributed on more than one island. ISSR fingerprints and sequencing of single copy genes indicate that radiation took place on each island separately, and morphological features used for species delimitation probably evolved independently several times. A microsatellite analysis using about 350 samples was performed to determine the amount of gene flow between species and populations. Additionally the extent to which similarity between species from one island in fingerprint pattern and sequence, is influenced by introgressions and hybridization should be tested. First results confirm our assumption on the radiation of *Micromeria*. In some cases it is indicated that former divided species were homogenised by hybridisation, so inter island colonisation might have occurred more frequently as indicated by molecular analysis.

P1617. Detecting a consequence of flowering-plant density on the inter-line crossing rate utilizing the F₁ seed production system of a brassicaceous vegetable

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F₁ hybrid seed is a main supply source of seed for vegetable production. These are produced by crossing of two different inbred lines as parents, which express heterosis, utilizing insect pollinators in field. It is often observed that the inter-line crossing rate fluctuates over years, and it may cause economic loss. In ecological studies of wild plants, it is reported that reducing seed production or lower outcrossing rate per individual plant tends to be observed in lower flowering-density or smaller population. Behavior of pollinator may be responsible for the phenomena. It appears that fluctuation of inter-line crossing rate in F₁ hybrid seed production is caused by the same mechanism of lower outcrossing rate in wild plant. The matter in F₁ seed production is equivalent to a major subject in plant ecology. Therefore, ecological study utilizing F₁ seed production system may be beneficial for both plant ecology and agricultural production. In this paper, we present our current study regarding a consequence of flowering-plant density on outcrossing, which utilized artificial population employing two inbred lines of *Brassica rapa*.

P1618. Cytogenetic and molecular characterization of *Narcissus x perezlarae* (Amaryllidaceae) and its putative progenitors

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Studies of ecological and evolutionary consequences of hybridization rely on accurate methods of hybrid identification. In *Narcissus*, with ca.70 species distributed in the Mediterranean area, several natural hybrids have been reported, among them *Narcissus x perezlarae*. Based on morphological and cytological characters, this taxon was proposed to be the result of interspecific hybridization between *N. serotinus* (2n=10, 30) and *N. cavanillesii* (2n=28). *N. serotinus* is distributed throughout the Mediterranean coast while *N. cavanillesii* is restricted to SW Iberia and N Morocco. The disjunct occurrence of *N. x perezlarae* (SW Iberian quadrant, N Morocco and Valencia in E Spain) suggests a polytopic origin and a potential evolutionary role beyond a sporadic occurrence. We report the chromosome number of *N. x perezlarae* (2n=29) in all the populations sampled, including those from Valencia, where one of the putative parents is absent. To verify its hypothesized origin, we also present preliminary data on sequence variation from four chloroplast regions (*ndhF*, *trnL-F*, *psbA-trnH*, *atpB-rbcL*) in a wide sample of wild hybrids and putative progenitors.

P1619. Hybrid speciation and evolution in UK *Sorbus* taxa

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Understanding the processes that have given rise to polyploid hybrid taxa is central to our understanding of plant evolution. Within the Avon Gorge, UK, there are three sexual diploid *Sorbus* species; *Sorbus aucuparia*, *S. aria*, and *S. torminalis*, one primary diploid hybrid, *S. x thuringiaca*, and five polyploid apomictic taxa (*S. bristolensis*, *S. wilmottiana*, *S. anglica*, *S. porrigentiformis* and *S. eminens*). In this study we are using a range of genetic markers (nuclear and chloroplast) in a population analysis to elucidate the origins and breeding systems of these Avon Gorge *Sorbus* taxa. To gain insights into hybrid formation, and determine the basis of the reproductive barriers that drive speciation, we are combining our molecular marker assays with a comprehensive study of breeding behaviour using controlled pollinations between the different taxa. An understanding of the genetic and reproductive relationships among the *Sorbus* taxa in this speciation 'hot-spot' will allow us to make recommendations for the conservation of this unique biodiversity.

P1620. Consequences of genetic introgression from foreign provenances into local plant populations

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Many restoration and habitat creation schemes involve sowing of seed mixtures to create species-rich communities. However, the geographical origin of the seed material used is still controversial. Besides the poor adaptation to local site conditions, one risk of introducing foreign populations is genetic introgression into native populations, which can reduce the fitness of locally adapted populations through outbreeding depression. We are studying the effects of gene flow from foreign provenances into local plant populations in *Lotus corniculatus* and *Plantago lanceolata*. Two generations of hybrids (F1 and F2) were made between local Swiss plants and plants of English and Czech origin, as well as plants from a contrasting habitat on a local level. The results of a field experiment, comparing the performance of the progeny of crosses between geographically or environmentally distant populations to that of within-population crosses, will be presented. Resistance of the hybrids to insect herbivores and pathogens will also be discussed.

P1621. Morphological variation in different generations of volunteer *Brassica rapa*

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The aim of this study was to compare variation in morphological traits of volunteer compared to crop plants of *Brassica rapa*, and to investigate if volunteer plants adopted a more weed-like morphology over time. We studied variation in morphological traits (flower size, hairiness and leaf colour) in a 5-year-old volunteer *B. rapa* population. Volunteer morphology differed significantly from that of crop plants, especially with regards to hairiness and leaf colour. Subsequently, *Brassica napus* and *B. rapa* were allowed to reproduce spontaneously in the field for one year after which morphological variation of the progeny was studied. The morphological variation in F1 plants appeared lower than for the volunteer *B. rapa*. A pilot microsatellite study showed indications of gene flow from *B. napus*. The combined effects of gene flow and local selection on the possibility for local adaptation in weedy populations will be discussed.

P1622. Asymmetrical crossability on interspecific cross and backcross between *Hemerocallis fulva* and *H. citrina*.

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Evolutionary outcomes could be affected by asymmetrical gene flow when young allopatric species come into contact and hybridize. The study of crossability on both interspecific cross and backcross is necessary to assess the asymmetrical gene flow. To examine asymmetrical crossability on both cross, we use *Hemerocallis fulva* and *H. citrina*, which are distributed in sympatry and sometimes hybridized on Hirado Island (Japan). Flowers of these species were hand-pollinated by conspecific, interspecific and F1 hybrid pollen. Seed set of interspecific cross was lower than that of conspecific when *H. fulva* was used as maternal plants, but no difference was found when *H. citrina* was used as maternal plants. Seed set of backcross was lower than that of conspecific cross irrespective of maternal species. Consequently, crossability was asymmetric when the two species were crossed but symmetric when pollen of F1 hybrids was backcrossed to either of parental species.

P1623. Characterization and identification of microsatellites in *Hemerocallis*

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Hemerocallis fulva (Liliaceae) and *H. citrina* show distinct pollination syndromes. *Hemerocallis fulva*, having orange perianth, is mainly pollinated by butterflies or bees and flowers exclusively in daytime, while *H. citrina*, having sweet-scented lemon-yellow perianth, is pollinated by nocturnal hawkmoths and flowers exclusively at night. Thus, co-flowering overlap, appearing in the morning and at dusk, is short in a day. Although these apparent ethological and temporal isolations limit gene flow between the two

species, hybrid populations are recorded in several localities in Japan. To evaluate gene flow in hybrid populations and between the two species, I developed microsatellite markers. Their level of variability and identifying polymorphisms will be evaluated and presented. Then, the frequencies of diagnostic alleles in one hybrid population will be evaluated.

P1624. Meet the ancestor: molecular data reveal that *Tragopogon porrifolius*, one of the diploid parents of *T. mirus*, may be polyphyletic

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Tragopogon mirus (Asteraceae) is a recently formed allotetraploid from North America; its diploid parents, *T. dubius* and *T. porrifolius*, were introduced from Europe in the early 1900s. ITS and ETS sequence data reveal that "*T. porrifolius*" comprises several genetically distinct lineages. The diploid "*T. porrifolius*" contribution to *T. mirus* appears in a different major clade of *Tragopogon* than other collections of *T. porrifolius*. This distinctive genotype is morphologically and karyologically distinct from other *T. porrifolius* and should perhaps be referred to as *T. eriospermus* (= *T. sativus*). This entity is widespread in North America and Europe (= cultivated salsify). Other subspecies of *T. porrifolius* (ssp. *australis*, ssp. *cupani*, and ssp. *longirostris*) appear in two closely related subclades. Sequence data indicate that *T. cupani* comprises both diploid and allopolyploid populations. Ssp. *longirostris* has also been introduced to North America. Thus, *T. porrifolius* is likely polyphyletic. Because of the vagaries surrounding ITS/ETS sequences, additional sequences are needed to test the extent of polyphyly of *T. porrifolius*.

P1625. An AFLP analysis for relationships of a putative hybrid species *Diplazium yakumontanum* and related species (Woodsiaceae).

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Interspecific hybridization is known to be a common phenomenon in vascular plants and provides variation that facilitates adaptation. *Diplazium* Sw. is a polymorphic fern genus including many hybrid taxa. Approximately 60 Japanese taxa have simple, simply pinnate to bipinnatifid, and bi- to tripinnate leaves, and putative hybrids among these taxa cause taxonomic confusion. *Diplazium yakumontanum* (tetraploid, 2n=164) is a Japanese endemic species and it is inferred from a hybrid species between *D. nipponicum* (triploid, 2n=123 and tetraploid, 2n=164) and *D. Petri* (tetraploid, 2n=164). The aim of this research is to study the genetic relationships between tetraploid hybrid species *Diplazium yakumontanum* and parental species, and find specific marker of these species using amplified fragment length polymorphism (AFLP). We discuss the utility of these specific markers for finding the origin of the genomes in the tetraploid hybrid species.

P1626. Hybridization between *Strobilanthes glandulifera* and *S. tashiroi* (Acanthaceae) in Okinawa Island, Japan

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Natural hybridization among two sympatric species of *Strobilanthes* from Okinawa Island, Japan was investigated. *Strobilanthes glandulifera*, which has a monocarpic life history strategy, and *S. tashiroi*, which has a polycarpic life history strategy, can be readily distinguished by morphological characters, including pollen morphology. However, natural populations in the Motobu Peninsula of Okinawa-jima, where *S. glandulifera* and *S. tashiroi* are found in sympatry, have characteristics that appear to be intermediate or intermixed between *S. glandulifera* and *S. tashiroi*. It is likely that these individuals originated through a cross between *S. glandulifera* and *S. tashiroi*. Sequences analysis of nuclear ribosomal internal transcribed spacer (ITS) regions and combined chloroplast DNA regions (*matK* gene and *trnL(UAA)-trnF(GAA)*, *trnT(UGU)-trnL(UAA)*, and *atpB-rbcL* spacer regions) suggest that putative hybrid originated from hybridization between *S. glandulifera* and *S. tashiroi*. This is the first report of a natural hybrid in the genus *Strobilanthes*.

P1627. Studies of *Potamogeton* hybrids identified in Polish populations using morphological, biochemical and molecular methods

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Clonal but also sexual reproduction, autogamy, lack of prezygotic isolations between species and capacity of long-distance spreading lead to the hybridisation within the genus *Potamogeton*. Once created hybrid often forms vigorous, long-persistent, clonal population. Some of them are partly fertile so introgression of genomic components from one taxon into another could be observed. Identification of *Potamogeton* hybrids based on the comparative morphology and anatomy solely is difficult and sometimes misleading, therefore it should be supported by biochemical or molecular data. Selected *Potamogeton* hybrids and their putative parents from Polish populations (both linear- and broad-leaved) have been studied by isozymes analysis, restriction fragment length polymorphism analysis (RFLP) of chloroplast DNA and of the nuclear rDNA ITS region to support the morphological and anatomical evidence. Methods are presented and the results are discussed.

P1628. Multidisciplinary evidence for differentiation-hybridization-cycles in the evolution of the polyploid complex *Achillea millefolium* agg.

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The polyploid complex of *Achillea millefolium* agg. consists of allogamous perennial herbs. It can be subdivided into 20-30 closely related taxa, growing in very diverse habitats of the Northern Hemisphere. Taxa form several reticulate lines ascending from diploids to high polyploids (2x - 4x - 6x - 8x). Representative samples (66 populations, 27 taxa) of the aggregate and its suspected hybrid links with other clades were studied with the help of DNA sequences (ITS, *trnL-F*), AFLP, plastid haplotypes (PCR-RFLP), cytogenetics, phyto-chemistry (essential oils, sesquiterpen-lactones), morphometry, etc. On the diploid level these data suggest general relationships and document basal genetic divergence and radiation. Polyploid taxa are of multiple and hybrid origin as revealed by specific AFLP bands and specific plastid haplotypes shared with their assumed diploid progenitors. The appearance of new specific AFLP and plastid markers in 4x-taxa signals new genetic differentiation and is followed by horizontal hybrid transfer between 4x-taxa. Comparable cycles of differentiation and hybridization are repeated on the 6x and higher ploidy levels.

P1629. Hybridization, introgression, polyploidization and the origin of the apomictic *Ranunculus cassubicus* complex

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The role of hybridization, auto- or allopolyploidy, and introgression of apomixis genes in the origin of apomictic complexes is still under debate. The *Ranunculus cassubicus* complex, comprising diploid or autotetraploid sexual and polyploid apomictic taxa, provides a model system for study. Isozyme, AFLP and SSR data clearly indicate an unique ancient hybrid origin of hexaploid apomictic *R. carpaticola* populations from NW. Slovakia, involving sexual autotetraploid *R. cassubicifolius* from the northern pre-Alps, and diploid sexual *R. carpaticola* from C. Slovakia. Although both parents occur mainly in forests, the apomicts occupy border zones and tend to establish in adjacent man-made meadows. Introgression from other distinct microspecies from meadows contributes to the origin of new genotypes and might confer on the hybrids a broader ecological tolerance than either parent. Polyploidy is a springboard for the origin of new hybrids, but appears to result in apomixis only in combination with hybridization. Apomixis, in turn, has permitted perpetuation and establishment of ecologically divergent hybrid genotypes.

P1630. Reticulate evolution in angiosperms: exploring network reconstruction methods.

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Reticulation, i.e. the occurrence of hybridization and introgression, is thought to be of major importance in the evolution of flowering plants. Since reticulate patterns cannot be reconstructed in a bifurcating phylogenetic tree, there is a need for methods that can reconstruct such patterns in angiosperms at the species-level. In this study we compared current available methods for network reconstruction, such as T-rex and NeighborNet. Using simulated data for these programs is not yet feasible, therefore their performance in detecting and representing hybrid taxa was tested using published 'real' DNA sequence data. The hybrid hypothesis in these studies was based on incongruence between different (DNA) data sets. We combined these data sets and compared the resulting output from the different network programs with the originally published trees. Also, we tested the output against our expectations about placement of a hybrid in a network. The question of how to interpret these networks in a meaningful biological way is discussed, as well as their added value compared with exploration of incongruent datasets using traditional phylogenetic methods.

P1631. Gene flow, hybridization, and divergence between Hawaiian *Dubautia* species

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This study used microsatellites and paternity analysis to estimate pollen movement at a fine scale between *Dubautia arborea* and *D. ciliolata*. A well-documented hybrid zone occurs in the transitional habitat of Waipahoehoe Gulch on the slopes of Mauna Kea, Hawaii, and contains plants that represent a continuous morphological spectrum between the parental species. The two species are fully interfertile and occur in parapatry, but maintain distinct morphologies and ecological specialization. A core region within the hybrid zone was designated and every individual plant mapped and sampled for genetic analysis. Seeds from 15 maternal plants were collected, germinated, and genotyped using eight microsatellite loci. Additionally, 30 plants from adjacent pure populations of the parental species were genotyped. Within the active hybrid zone, mating appeared to be random with respect to plant phenotype, generating a wide array of intermediate forms. Therefore, gene flow was expected to follow an isolation by distance model. Results are interpreted in light of the role of hybridization in ecological divergence and the evolution of adaptive traits.

P1632. Hybridization of *Goodyera chejuensis* (Orchidaceae) inferred from Molecular evidence

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Goodyera x chejuensis S. Kim, a recently described orchid endemic to Korea, shares many morphological characters and grows in a sympatry with both both *G. schlechtendaliana* Reichb. fil and *G. velutina* Maxim., raising the possibility that *G. x chejuensis* was originated from hybridization of *G. schlechtendaliana* and *G. velutina*. To verify the hypothesis, the ITS sequence of nrDNA, and psbA-trnH, trnSG and trnL of cpDNA for 32 accessions from 6 taxa of *Goodyera* were analyzed. In the ITS regions of *G. x chejuensis*, four single nucleotide polymorphisms sites were observed, at positions 102 (G/A), 440 (A/G), 464 (C/T), and 642 (A/T). Of these, *G. x chejuensis* shares species-specific character states with *G. schlechtendaliana* at three sites, positions 102 (G), 440 (G), and 464 (C). Also, *G. x chejuensis* shares two species-specific character states with *G. velutina* at positions 653 (A) and 512 (T) in the ITS region. *Goodyera x chejuensis* and *G. schlechtendaliana* share 10 and 39 species-specific character states in trnH-psbA and trnSG of cp DNA, respectively. Our molecular data suggest that *G. x chejuensis* was originated from hybridization between *G. schlechtendaliana* and *G. velutina*.

P1633. Hybrid origin and expansion of tetraploid *Achillea* taxa from E Asia to N America: DNA-based, morphological and ecogeographical evidence

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A group of 2x- and 4x-taxa of herbaceous perennial *Achillea* (Asteraceae) in E Asia has been studied by DNA-analyses (sequencing, AFLP, cpDNA PCR-RFLP) and other multidisciplinary methods. *A. acuminata*-2x belongs to *A. sect. Ptarmica*. *A. asiatica*-2x is an eastern member of the *A. millefolium* aggregate in *A. sect. Achillea*. *A. alpina*-4x and *A. wilsoniana*-4x form a nearly continuous morphological, ecological, and geographical link between the two very different diploids. A NJ genetic distance analysis of AFLP data clearly demonstrates an intermediate position of the 4x- between the two 2x-taxa. 5 plastid haplotypes were detected by PCR-RFLP analyses in these species. *A. alpina*-4x and *A. wilsoniana*-4x share one haplotype only with *A. asiatica*-2x. On the basis of all these evidence and previous cpDNA *trnL-F* and nrITS sequence data, the two allotetraploids are regarded as products of a possibly Pleistocene hybridization between an *A. acuminata*-like ancestor as father and an *A. asiatica*-like as mother. This was followed by considerable secondary genetic diversification on the 4x level and expansions from Asia into the large area of northern N America.

P1634. Investigating ancient homoploid hybridisation patterns within the genus *Diphasiastrum*

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Several defined species within the genus *Diphasiastrum* are considered to be of hybrid origin. These assumptions are primarily based on observations of populations with intermediate morphology and high rates of spore abortion in both Europe and North-America. Cytological and molecular support for these theories is, however, lacking. The main goal of this project is to investigate and estimate the frequency of speciation through hybridisation within this genus. This primarily implies investigating congruence between cpDNA - and nuclear single copy gene sequences (LEAFY, RNA polymerase and phosphoglucose isomerase, PGI) to identify parental lineages of the putative hybrid species. As *Diphasiastrum* is known as a genus with notoriously uncountable chromosomes, assessing ploidal level is crucial to define what kind of hybridisation events that have occurred, i.e. if the hybrids are either homoploid or allopolyploid. Such data will be obtained using Quantitative Feulgen photometry. Furthermore, microsatellite - and allozyme markers will be used for a more detailed study at population level.

P1635. Reproductive isolation in Mediterranean deceptive orchids

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The maintenance of species boundary among related sympatric species is clearly dependent on the strength of reproductive isolation. Orchid flowers are often highly specialised to attract and fit their pollinators, suggesting a prominent role for pre-mating reproductive isolation in maintaining species boundaries. To investigate the presence and the strength of post-mating barriers in Mediterranean orchids we performed a large number of interspecific crossing experiments and analysed fruit set production and seed viability. Then we tested the relationship between reproductive isolation and genetic distance for post-mating pre-zygotic barriers and for post-zygotic barriers. Results clearly indicate that post-mating barriers (both pre-zygotic and post-zygotic), including late post-zygotic barriers, as hybrid sterility, largely contribute to prevent gene flow among co-flowering food deceptive orchids with a generalized pollination compared with other orchid groups with an highly specialised pollination biology like the sexually deceptive *Ophrys*.

P1636. F1-dominated hybrid zones: can genome-habitat interaction alone maintain species barriers?

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F1-dominated hybrid zones (**F1DZs**) are hybrid zones in which F1 hybrids are highly fertile and make up most or all of the hybrid population. In such cases hybrid zone population structure varies dramatically according to habitat - in undisturbed habitats intermediate between those of the parents, most or all hybrids are F1s; where the habitat is disturbed or otherwise atypical, most hybrids are post-F1s. Because post-F1s are required for gene flow between the parents, this implies that genome-habitat interaction maintains species barriers in F1DZs. F1DZs were probably far more common before human interference began. Hybrid zones between *Rhododendron ferrugineum* (Acid soils) and *R. hirsutum* (Basic soils) are unique among published examples: F1s occur intermixed with both parents and a very few post-F1s on a mosaic of soil types. Here soil pH appears to determine where each genotype class grows. Implications for how species barriers might arise and be maintained are discussed.

P1637. Evolution of *Cuphea* spp. during domestication

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Cuphea, a semi-domesticated crop, is a potential source of oil suitable for industrial uses. Indeterminacy, seed shattering and post-harvest dormancy constitute its domestication syndrome. PSR23, a selection from a *Cuphea viscosissima* Jacq. x *Cuphea lanceolata* f. *silenooides* W.T. Aiton cross, was assessed for seed, capsule, and plant morphological and physiological characters. PSR23 germplasm produced during six consecutive years showed a reduced genetic diversity favoring characters better suited to cultivation. Morphological and physiological divergence of PSR23 from its wild progenitors increased as indicated by consistent and gradual differences in the principal components structure, and by smaller fractal dimensions of seeds, capsules and mature plants. Directional selection through agricultural practices increased the frequency of tall and more erect genotypes, heavier and more circular seed with reduced post-harvest dormancy and higher germination index as compared with the wild progenitors. Indehiscence and determinacy are critical to fully domesticate PSR23. Stabilizing selection will be needed to maintain the domesticated crop.

P1638. Investigation of durability of four types of natural fibre-polyethylene composites against the fungus *Coriolus versicolor*

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In order to study the durability of four types of natural fiber-polyethylene composites containing kenaf, rice hulls, newsprint and wood flour as the reinforcement at 25% and 50% by weight against *Coriolus versicolor*, available specimens of the composites were sampled.

After preparing the specimens and culture medium (Malt Extract Agar), specimens were contaminated with cultured fungus (laboratory conditions 22C, 75% relative humidity) for fourteen weeks. After this period, weight reduction, water absorption of specimens were measured and compared with control samples. The results showed that weight reduction of samples after contamination with fungus was significant. The composites containing 50% uncompatibilized wood flour had the highest weight reduction and the composites containing 25% with 1% compatibilized wood flour had the least weight reduction as compared with other types of composites.

The highest Water absorption of samples, after contamination with fungus has been observed in the composites containing 50% uncompatibilized wood flour and the composites containing 25% with 1% compatibilized wood flour had the least water absorption.

P1639. Natural hybridization in *Betula* in Iceland

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Betula (birch) is a genus of some fifty species distributed throughout northern temperate regions. In Iceland, two *Betula* species coexist, *B. nana* (2n=28, diploid dwarf birch) and *B. pubescens* (4n=56, tetraploid downy birch). Downy birch is the major tree species forming natural woodlands in Iceland, which today cover only one percent of the total land area. The morphology of Icelandic birch is extremely variable, evidently due to introgressive hybridization. Such introgression (gene flow) could only occur via interspecific hybridization and repeated backcrossing of the hybrids. Flowering periods of the two species overlap significantly in Iceland, due to a short growing season, allowing interspecific hybridization to take place in birch. Our result show that about 10% of examined birch plants are triploid hybrids (3n=42). This is a much higher frequency than has been expected. The populations showed continuous morphological variation connecting the species, but karyotypically they consisted of only three types of plants, diploids, triploids and tetraploids. No aneuploids were found. Some of the tetraploid plants had *B. pubescens* morphology as expected, but most of them had intermediate characters. Most of the diploid plants had *B. nana* morphology, but some were intermediates and a few had *B. pubescens* morphology. The triploid plants were either intermediates or they resembled one of the two species. Introgressive hybridization in birch is clearly a continuing process, probably as a means of survival in a difficult environment. The aim of this research is to evaluate the extent and the possible role of natural hybridization between *B. pubescens* and *B. nana* in natural woodlands in Iceland.

P1640. The genetic variation of *Licuala* and *Cyrtostachys*, *Palmae*, in the Tasek Merimbun Heritage Park in Borneo Island.

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Licuala is a fan-leaved palm and *Cyrtostachys* is a feather-leaved palm that distribute mainly in South East Asia. We studied their RAPDs patterns in the northwestern part of Borneo Island. The aim is to know the genetic variation of their populations in the natural habitats. Leaves of 168 plants of *Licuala* and 189 of *Cyrtostachys* were collected in the Tasek Merimbun Heritage Park in Brunei Darussalam. Genomic DNAs were extracted and the DNA segments were amplified by using six oligonucleotide primers. The cluster analysis was made by using data of the absence-presence of electrophoretic bands. As a result, 115 polymorphic fragments were amplified and two major clusters were recognized in *Licuala*. One cluster consisted of *L. paludosa* Griff and the other was an unknown taxon. In *C. renda*, 74 polymorphic fragments were recognized. Populations showing wide genetic variations located in a forest that included complicated microhabitats with swamps and dry slopes.

P1641. Molecular markers as a tool for identification plant dispersal in river corridors: An example of *Sparganium erectum*

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Rivers represent linear corridors in landscape. All possible suitable sites for water plants are connected by the stream flow and species can migrate along the linear structure downstream. Transport of both vegetative and generative diaspores is possible. But it is still the question how numerous and how far diaspores can be dispersed.

We used AFLP markers to distinguish vegetative and generative dispersal, and to identify long-distance dispersal in river system. 258 samples from 66 populations of *Sparganium erectum* from the two river basins in Czech Republic were analysed. Based on AFLP analysis we can state the following: (1) vegetative dispersal is more frequent in lower parts of rivers, (2) generative dispersal is important factor in establishment of new localities, (3) populations in lower parts of rivers are genetically more diverse, genotypes are dispersed downstream, (4) more than a half of total variability is

found within populations, (5) low correlation between genetic and geographic distance implies strong effect of water streams in dispersal processes of river plants.

P1642. Differences among European populations of *Silene latifolia* for sexually-dimorphic traits in response to water availability

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To understand evolution in natural populations it is important to determine the ecological factors, e.g., water availability, that contribute to population differentiation. In this study we grew five European *Silene latifolia* populations in two moisture treatments to determine if they differ for floral, physiological, and leaf traits. A French population had many small flowers, an effect especially pronounced for males. In contrast, a Czech population had few very large flowers. The small-flowered French population dramatically increased flower number in the moist treatment from the dry treatment while the other populations had modest increases. This population also had the highest water use efficiency (WUE; which was correlated with leaf thickness) across both treatments. The large-flowered Czech population had high WUE in dry conditions but low WUE in moist conditions, which suggests that the population can alter WUE but continue to produce large flowers, which are generally considered costly. The differences between populations for WUE may be the result of contrasting patterns of selection on leaf traits via differences in water availability.

P1643. The maintenance of cyto-nuclear gynodioecy in wild radish (*Raphanus sativus*): relative seed fitness between females and hermaphrodites based on molecular genotyping

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In gynodioecious breeding system, sex determination often involved cytoplasmic male sterile (CMS) genes and nuclear genes restoring male function. In *R. sativus*, Ogura cytotypic is responsible for CMS and has been well analyzed at molecular level. Thus, whether hermaphrodites have a CMS gene or not is easily surveyed using an Ogura-specific marker. To test the theoretical prediction that females should have at least a slight female advantage over hermaphrodites, I compared seed fitness among the three genotypes: females, hermaphrodites with CMS gene and those without it. Neither many quantity parameters nor seed germination rate differed among the genotypes. In fruit set and total weight in fruits and seeds, hermaphrodites without CMS gene achieved significantly large values than the females. These results suggest no seed fitness advantage of females in *R. sativus*. However, I found females invested larger proportion in fruit and seed production than hermaphrodites despite their small plant biomass. If there is a metapopulation structure including populations where females grow as much as hermaphrodites, cyto-nuclear gynodioecy is to be maintained.

P1644. Effect of predispersal seed predation and population size on population dynamics of two species of genus *Aster*

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European landscapes are highly fragmented. As a result many plant and animal populations are small and isolated. Isolation and small population size can have negative effect on reproduction and survival of populations due to e.g. low pollinator attraction. On the other hand, small population size can have also positive effects such as lower attraction of herbivores and thus lower herbivore damage.

We studied relationship between population size, plant performance and herbivory using species from genus *Aster* and its monophagous herbivores from genus *Coleophora* as model species. To test dispersal ability of the herbivore, we attempt to estimate genetic relatedness between the herbivore populations. The results show higher density of the herbivore and consequently higher seed damage in larger populations. This effect is however compensated by higher seed production in larger population resulting in non-significant relationship between population size and number of intact seeds.

P1645. Ecological and genetic differentiation in an endangered aquatic species *Nymphoides coreana* (Lev.) Hara in Japan

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Nymphoides coreana (Lev.) Hara is an endangered aquatic plant and now its distribution is geographically isolated by the loss of many populations. The species has proven to be an inbreeder by autogamous pollination. The obligate selfing and geographical isolation may lead to the genetic and ecological differentiation among populations. Our field survey and cultivation experiments have shown that the populations in the paddy fields are annual and, on the other hand, those in irrigation ponds are perennial. Flower size was the smallest but the size and weight of the seeds were the largest in the plants from paddy fields. Germination rate of the seeds was the highest under light and anaerobic conditions in the plants from irrigation ponds but it was highest irrespective of light condition under anaerobic condition in the plants from paddy fields. Allozyme analysis has also shown the occurrence of distinct geographic variation among the populations of *N. coreana*. These results show that some ecotypes occur within the populations of *N. coreana* in Japan.

P1646. Genetic diversity at the northern edge of the distribution of *Anthyllis montana* ssp. *jacquinii* (Fabaceae)

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Taxonomically, the mountain species *Anthyllis montana* L. might be best treated as two subspecies with a western and eastern distribution area in Europe, respectively. As part of the eastern subspecies *jacquinii* a small number of populations in Austria is at the northern-most limit of the species' distribution. These populations are highly isolated, patchy structured, to some extent individual-poor, and as a consequence thereof, are considered to be threatened. Seven populations of *Anthyllis montana* in Austria went already extinct.

For conservation management, a molecular analysis utilising AFLP fingerprints and chloroplast PCR-RFLPs will be performed to characterise levels of genetic diversity. The remaining Austrian populations have been sampled and will be compared to more southern (i.e. "central") populations from Slovenia in a hierarchically structured approach including subpopulational patches. Correlations between genetic diversity and population size will be tested to uncover Austrian populations which might be most critically endangered.

P1647. Demography of *Betula fruticosa* Pall. in dependence from types of habitat, Vitimskoye upland, East Siberia

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On the Vitimskoye upland one of the basic dominant of shrub vegetation is *Betula fruticosa* Pall.

The extremely continental climate and continuous spreading of permafrost are determining factor in forming of ecological conditions for *Betula fruticosa*.

In demography structure the maximal number of specimens of generative age have been marked in all coenopopulations of *Betula fruticosa*. The large number of pregenerative specimens of *Betula fruticosa* have been marked in communities where the frost cracking is observed. The sites of naked ground, which are basic microecotope for regeneration of *Betula fruticosa*, are formed there. The limiting factor for regeneration of *Betula fruticosa* is supermoistening or lack of moisture. For example, in community are located to negative forms of a relief with the difficult drainage, and in community are located dry southern slopes, quantity of young specimens very low.

P1648. Preliminary insights in demographic monitoring of the endangered woody Cruciferae *Vella pseudocytisus* in Spain

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The scrubby member of the Cruciferae family *Vella pseudocytisus* L. is endemic to the Western Mediterranean basin and consists in two subspecies in peninsular Spain: *pseudocytisus* and *pau*

Gómez Campo. Both taxa live under continental climate, on gypsum soils and their habitats range from open semi-natural steppic scrublands to artificial slopes between cereal croplands. Their extant populations have been fragmented and equally catalogued as Endangered in the recent Spanish Red Data Book, in stead of they were protected in their corresponding regional laws of plant conservation.

From 2001 demographic monitoring has been developed in several locations of both subspecies, distant 300 km apart. Their futures seem to be compromised as a slight decline has been projected for both. The nearly absent recruitment and the reduced survival of the juveniles observed are commented taking into account the high germination rate experienced in the lab. The possible effect of the past cycle of dry (*bad*) years is discussed taking into account differences both in regional conditions and precipitations regimes.

P1649. Variability of one year old seedlings of *Pinus peuce* Griseb.

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Seedlings of Macedonian pine were obtained from stored seed harvested in two successive years from artificial forest plantation near Ivanjica, Serbia. The seed was sowed in containers in mixture of soil, peat and sand after standard presowing wet-cold treatment (six months at +4°C in refrigerator). Differences between measured characteristics of one year old seedlings (the length of main root, the length of all roots, the mass of air-dried roots, the root collar diameter, the height of seedling, the length of primary needles and the length of secondary needles and the mass of air-dried green part of seedlings) according to age of seed harvesting and according to individual trees were discussed. These results were compared with those obtained from two weeks old seedlings of the same origin (trees). Differences between half-sib lines were proved by statistic methods (descriptive sta., anova).

P1650. Investigations of *Helleborus purpurascens* Waldst. & Kit. (Ranunculaceae) in Ukraine.

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H. purpurascens is the East-Carpathian species, the tertiary relict, endemit, for which the natural rarity is proper. The plant has medicine properties due to contains of cardioglycosides, which have a cardiotonic effect.

The investigations on onthomorphogenesis features, natural populations structure and chemistry (for a contains of toxic metals and microelements) were conducted.

At the first an investigation of natural grow places of *H. purpurascens* on the population level was conducted in Ukraine.

There were studied mountain and lowland populations of *H. purpurascens*, which are on the distribution limit within Ukraine.

On the way of population investigations main population parameters, such as area dispersal, number, age individual status, age spectres character, onthogenesis features, vitality, reproduction and restoration, phytomass storage, were studied. Changes of these parameters and population strategy were studied too in the antropogenic pressure conditions.

A chemical monitoring of heavy metals contains in the plant matherial - roots with rhizoma from diverse natural grow places (mountain and lowland) is accompanied.

P1651. Demographical structure of populations of *Adenocarpus gibbsianus* Castr. & Talavera (Leguminosae). Influences of the reproductive success.

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Adenocarpus gibbsianus Castr. & Talavera (Leguminosae) is an endemic species occurring in SW Spain paleodunes that shows only 3 populations as well as some scattered individuals. This so low number of populations makes this species very sensitive to any instability in their demography. To assess their structure as well as their regeneration capacity, and so the stability of this species, we counted the number of individuals belonging to the seedling, vegetative and reproductive stages in every population as well as their change in three consecutive years. Two populations showed an apparently stable structure whereas very

little recruitment was observed in the third one. Additionally we tried to guess if a differential reproductive effort, estimated as the number of seeds produced, could explain these differences but no apparent relationship was found. So, whereas two populations looked stable, the other one behaved as a fossil population in spite of its important reproductive potentiality, suffering strangulation in the younger stages. To avoid wasting resources, *in situ* conservation of this species should pivot on the most stable populations.

P1652. Population biology of herbaceous plants infected by systemic fungal pathogens

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The range and dynamics of natural plant populations can be significantly influenced by interactions with other trophic levels such as herbivores and pathogens. The latter has been brought into focus only in last few years, but the results are striking. The systemic fungal pathogens can influence their hosts in various ways, e.g. by altering growth, fertility, interactions with herbivores etc.

To broaden this knowledge, I started to investigate two systems - *Scorzonera humilis* with its smut fungus *Ustilago scorzonerae* and *Falcaria vulgaris* with its rust fungus *Puccinia sii-falcariae*. The study includes field observations of populations, in which both healthy and diseased plants were marked, measured and will be followed for several years. The data are going to be processed using matrix modelling to find the most sensitive stages in life cycle of the host under pressure of the pathogen. There will be also greenhouse experiments carried out to verify and explain the outcome of the field observations. The poster is aimed to present some of my first year's data on *Falcaria vulgaris* and prospects of the work. I hope to get answers for some of still unanswered questions.

P1653. Demography of *Dioon edule* in central Veracruz Mexico

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The demographic dynamics of one well conserved and two fragmented populations of the cycad *Dioon edule* Lindl (Zamiaceae) were studied using projection matrix modelling. Density and spatial distribution patterns differed between populations according to seed dispersal traits. In all scenarios the population growth rate (λ) was most sensitive to changes in the abundance of adult plants. The elasticity reproductive component (F) for the three populations was zero and stasis values (L) were higher than expected. *Dioon edule* behaves like a tree life-form species according to Caswell. The specie's response to different management conditions suggests that the conservation of adult plants is critical for the species survival. This may be true for all cycad species. It was detected that habitat disturbance influences the population dynamics of *D. edule*, especially in the persistence of adult plants. The results are discussed with vision for practical conservation management.

P1654. Maximum entropy method as a tool for exploring spatial population structures

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The spatial probability distributions of shoots in plant communities were modeled. The vegetation potentials at a given point depend on the distance of the nearest individual of species i :

where s denotes the number of occurring species. We suppose the spatial means of these remain constant. The vegetation is in a stationary state if the corresponding distribution $p(\underline{v})$ is the most likely, namely realized by the most of the possible configurations.

The method was applied to a Bottle Sedge stand (*Equiseto limosi-Caricetum rostratae*) and a Slender Sedge stand (*Caricetum elatolasiocarpae*).

The distribution of *Carex rostrata* was independent on that of *Equisetum fluviatile*. In the case of *Carex rostrata* we obtain the following probability density function for :

where c is the lattice constant within tufts.

This means that the sedge shoots take place according to a Poisson distribution on a fractal of dimension $D = 1.5494$. The

Lagrange parameter = 0.18166 is proportional to the shoot density on the fractal.

For *Carex lasiocarpa* we have similar results but this species interacts with *Carex elata*. This causes some distortion in spatial structure, accounted for by the model.

P1655. Is dormancy induced by stress?

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Dormancy is a poorly understood life history phenomenon in which a mature perennial plant fails to sprout any aboveground tissue in one or more growing seasons. It occurs among several plant families, but generally occurs in rare plants with difficult growing requirements, most often described in orchids. As such, experimental studies to address the nature of this condition have so far been lacking. We defoliated and shaded individuals of *Cypripedium calceolus* and *Cephalanthera longifolia* in five populations in Estonia in 2002 and 2003 to ascertain whether dormancy is a potentially adaptive means of dealing with environmental stress resulting in low photosynthesis. Sighting data was analyzed with mark-recapture statistics in order to estimate dormancy and survival likelihoods, otherwise not estimable in an unbiased fashion.

Dormancy appears an adaptive condition, allowing the plant to buffer environmental stress without increasing mortality risk, though the differences in response to treatment suggest that dormancy may have a different life-history context for each species.

P1656. Population structure of *Apium repens* in Belgium

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Apium repens (Jacq.) Lag. is a low creeping perennial umbellifer with a mainly European distribution, that is rare throughout its distribution area. It has been included in the European Habitat Directive 92/43/EEG as wild species that should be protected integrally in the European Union. It has been found to subsist in Belgium in five populations of very unequal size and in different habitat types (Ronse & Vanhecke 2004). In four populations a demographic study has been carried out since 2003, with monthly measurements of individual plants during six months each year. This poster presents results concerning the population structure of *A. repens* at different locations and its evolution with time. Moreover, the influence of some climatic and other abiotic variables on population structure and on reproduction is investigated.

References

Ronse A. & Vanhecke L. 2004. The conservation biology of creeping marshwort (*Apium repens*) in Belgium: aims, methods and first results. *Scripta Bot. Belg.* 29: 147-150.

P1657. Demographic structure of fern populations in South Siberia

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Population demography of 6 forest and 4 rocky ferns was studied. The results reveal, that the long-term existence and stability of fern populations in native conditions is ensured by coexistence of gametophyte and sporophyte subpopulations, duration of sporophyte life and sporophyte ability to vegetative propagation. The main peculiarity of the forest fern populations is ecological separation of gametophyte and sporophyte subpopulations. Gametophyte subpopulations of *Dryopteris expansa*, *D. carthusiana*, and *D. filix-mas* develop on rotten windfall; sporophytes of these species are able to effective vegetative propagation by young offsprings. Gametophytes of *Athyrium distentifolium*, *A. filix-femina*, *Phegopteris connectilis* and *Gymnocarpium dryopteris* prefer the naked plantless soil. Sporophyte subpopulations demonstrate a complex age composition of individuals, gametophyte subpopulations have a complex sex structure. Gametophytes and sporophytes of the rocky ferns (*Cryptogramma stellerii*, *Cystopteris fragilis*, *Polypodium sibiricum*, *Asplenium ruta-muraria*) grow together and do not form as a rule separate subpopulations.

P1658. How does a gypsophyte reproduce? Factors controlling the reproductive output of *Helianthemum squamatum*.

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We have examined the relationships among flowering phenology, plant size and reproductive output in the endemic Iberian gypsophyte *Helianthemum squamatum*, in two contrasting environments (north and south slope aspects) for a two-year period.

Survival rate of reproductive plants was less than fifty per cent, but plants persisting the second year increased noticeably their fruit and seed production. First flowering and peak flowering dates happened earlier in the south-facing slope, where flowering intensity and synchrony also were higher. Plants reproductive behaviour not only was different in the two studied environments but it also varied between years.

Our results suggest that microevolutionary processes may be occurring in this species as a consequence of the pronounced environmental heterogeneity.

This kind of studies may help to clarify some aspects of gypsum community strategies and, they also can improve our knowledge about the potential response of this systems to climate change.

P1659. The effects of grassland management on the growth and viability of populations of the endangered, long-lived plant *Scorzonera humilis*

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We studied growth and viability of five populations of the long-lived plant *Scorzonera humilis*, a characteristic species of wet, nutrient-poor grasslands in relation to agricultural intensification. To investigate the effects of fertilisation and different cutting regimes on the population viability of *S. humilis* we carried out an experiment that simulated different agricultural practices at one study site. Projections based on mean transition matrices over several years indicated that populations at nutrient-poor sites would grow ($\lambda > 1$), whereas populations at nutrient-rich sites would decline continuously ($\lambda < 1$), if conditions stayed the same. Stochastic simulations showed that the extinction risk for a medium-sized population at a nutrient-poor site is very low, whereas the long-term survival even of large populations at nutrient-rich sites is unlikely if favourable conditions for recruitment are not restored. The experiment showed that a combination of fertilizing and early cutting drives populations quickly to extinction.

P1660. Ramet demography of *Allium oleraceum* (Liliaceae), common European geophyte

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Allium oleraceum is a perennial geophyte with annually renewed bulb. Sexual reproduction is extremely rare but species spreads via underground daughter bulbs and bulbils within inflorescence. Demographic data were collected from three populations that occurred in contrasting habitats (forest, scrub and rocks) for 6 years. Ramets were classified both by their size and flowering status. Overall, the population growth rate (λ) ranged from a low of 0.45 to a high of 1.27. Abortion rate of flower scapes was high in forest population (90-100%) but lower in other populations (30-70%). Populations showed unimodal population structures but were dominated by different stages - sterile ramets of a middle size or juveniles. Elasticity values associated with reproduction (bulbils+seeds) contributed to 3-11% of λ and were more important in less shaded populations. Although stasis and progression transitions made approximately equal and dominant contributions to λ (40-56%), progression was more important in less shaded populations. Clonal growth via daughter bulbs contributed more to λ in forest population (5%) than in other ones (less than 2%).

P1661. Population dynamics of *Abies hickelii* (Flous et Gausson) (Pinaceae), an endangered species exposed to burning and harvest on the Pico de Orizaba Mountain, Veracruz, México.

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Abstract

Abies hickelii is a fir species endemic to Chiapas, Oaxaca and Veracruz; its area of distribution is used for grazing and logging. To assess if the species is truly endangered we study the population dynamics of *A. hickelii* on the Pico de Orizaba Mountain, Veracruz using Lefkovich transition matrices. Data on saplings, juveniles, adult trees and fecundity, were taken from six permanent 1000 m² plots. λ was 0.99, indicating that the population is very close to numerical equilibrium but decreasing, so the species could be considered as endangered; the stages at which is most sensitive are Juvenile II and Adult I. The GLF demographic triangle indicates that the species invests 99% of elasticity in survival. Effects of fires and harvest on trees were simulated for different persistence, transition and fecundity probability values. λ is quite sensitive to changes in Juvenile II tree survival and harvest, making this stage the bottleneck for population growth. Survival makes the greatest contribution to λ . Reproductive rates concentrate in the adult stage. Logging reduced transition rates from juveniles to adults and had the greatest effect on λ .

P1662. Age structure and vitality of *Dentaria quinquefolia* M. B. coenopopulations in phytocoenosis of the Mountainous Crimea

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Dentaria quinquefolia M.B. coenopopulations were studied in 3 areas covered by Querceto- Carpinetum mercurialidosum association: 1 - without anthropogenic press; 2 - collection for bunches; 3 - recreation, plant collection, pasture. In the Area 1, age spectrum is complete; juvenile, immature, virginil specimens give in total 52.2%, generative 39.8%, senile 8.0%. Plant height is 43.2 ± 0.8 cm, inflorescence length is 8.9 ± 1.4 cm, leaf length is 33.4 ± 0.9 cm. In the Area 2, pre-generative specimens contain 21.4%, generative 41.7%, senile 36.9%. Plant height is 29.4 ± 1.2 cm; inflorescence length is 5.1 ± 1.7 cm; leaf length is 22.4 ± 1.8 cm. In the Area 3, only subsenile (40%) and senile (60%) plants grow; height is 20.7 ± 1.3 cm, leaf length is 14.5 ± 1.8 cm. Anthropogenic press has destroyed the age structure of the coenopopulation, suppressed vitality and self-sustaining ability. The degree of destruction of nature population of *Dentaria quinquefolia* M.B. depends on quantity of anthropogenic press.

P1663. Fertility analysis of *Vriesea gigantea* (Gauldchaud, 1864), Bromeliaceae.

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The breeding system and fertility are related with genetic diversity of natural populations. Little is known about reproductive biology of bromeliads populations. This study aims to assess plant fertility and seed viability of *Vriesea gigantea*. Four populations from south of Brazil have been investigated. In each population five plants (mother-plants = MP) were sampled. From each MP 90 seeds were used for fertility analysis. Seeds were sterilized and placed on ½ MS medium with B5 vitamins. Plant fertility were assessed considering: (a) reproductive potential (total number of flowers per plant); (b) fruits production (% fruits = total number of fruits per plant/total number of flowers per plant x 100); (c) the number of seeds per fruit were estimated from 5 fruits of 10 MP sampled randomly. Data were analyzed using ANOVA and means compared by Tukey test. Seeds were mostly viable: after 15 days 94% were germinated. The mean of flowers per plant were 155 (ranging from 45 - 267), the fruit production ranged from 6.3 to 95.5%, and the number of seeds per fruit ranged from 0 to 708. The results showed significative differences among plants and populations.

P1664. Morphophysiological dormancy, germination and recruitment of *Leucopogon* (Ericaceae): implications for functional types and species persistence in a fire prone region

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In fire prone regions, obligate seeding species depend on regeneration from seed, and seedling recruitment is a particularly critical phase for their persistence. To maximise recruitment, obligate seeders are assumed to primarily have dormancy related to fire cues. This study, conducted in southeastern Australia, investigated the dormancy and recruitment of obligate-seeding *Leucopogon* shrub species, including the threatened *L. exolasius*. All species had morphophysiological dormancy, which was not broken by direct fire cues. However, a post-fire flush of emergence occurred and was always restricted to autumn, irrespective of the timing of the fire, or that rainfall is aseasonal. Seedling growth was significantly reduced for delayed emergents. These results show that *Leucopogon* germination and recruitment could vary considerably in response to the fire regime, compared to other species within the same functional group. There are likely to be many other morphophysiological dormant species in this, and other, fire-prone regions. It is possible that these species may tolerate fire, rather than be specifically adapted to it.

P1665. Recolonization potential of bryophyte fragments in hydrologically restored rich fens

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Fragmentation (mainly due to drainage) of rich fen patches in the landscape may cause dispersal limitation for plants occurring in the biotope. Specific bryophyte species are often frequent and abundant in rich fens with undisturbed hydrology. For these reasons we initiated a reintroduction study, using fragments of *Campylium stellatum*, *Pseudocalliergon trifarium*, *Scorpidium cossonii* and *S. scorpioides*, in combination with different kinds of soil disturbances and treatments (such as mowing, addition of lime etc.). Desiccation protection was also tested. The study was performed in a hydrologically restored rich fen. To evaluate the growth potential of the gametophyte fragments a greenhouse experiment was performed. Comparison was made to evaluate fragment establishment and growth potential of the four moss species, which represent different growth forms. Fragments of different parts of the gametophyte were tested. Preliminary results show similar responses in all four tested species, with a higher survival rate in the greenhouse experiment compared to the field study and a positive response in growth and survival rate with addition of lime.

P1666. Seed germination in *Mangifera indica* Haden and Manila varieties.

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Haden and Manila are monoembryonic and polyembryonic varieties of mango respectively. In Manila variety one zygotic embryo and several nucellar embryos are present in the same seed. To compare germination capacity of these different embryos, mango fruits were collected in Sinaloa, Mexico during June of 2003 and July 2004. The seeds were characterized and sowed at $25 \pm 2^\circ\text{C}$ with photoperiod 12/12h. Radicle's protrusion was daily quantified. The germination processes takes in 16 days. Seed germination occurs 3 to 5 days earlier in Haden than in Manila. In 2003 sample, 100% of Manila seeds germinate, at least one embryo germinating from each seed. In 2004 germination capacity was 60%. In samples of both years there were one to five germinated embryos by seed. In the other hand germination capacity in Haden was 75% and 99% respectively, with only one embryo germinating by seed. Differences in germination capacity were discussed taking account differences in seed characterization and harvest periods. Identification of genetic origin of embryos could be useful to evaluate the vigor value and to make recommendations for seedlings establishment.

P1667. The effect of fragmentation on the soil seed bank of dry meadows

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One of the main threats of dry meadows is their isolation. Hence, we investigated the effect of fragmentation on the seed bank. Our study sites were six slopes between terraced vineyards covered with dry meadows. For the soil seed bank investigation we used the seedling emergence method. The average seed density was very low (max. 5556 seeds/m²). We compared the vegetation and the seed bank by using the Sørensen index (SI). Although the vegetation of all slopes is similar (SI = 0,45 to 0,80), the seed banks of the slopes show differences (SI = 0,21 to 0,59). Comparing the vegetation with the seed banks of the same slope, we found low similarity, too (SI = 0,22 to 0,40). While the similarity of the present vegetation is a result of nowadays management, differences in the composition of the seed banks might be caused by the history. For the small seed density and the difference between the vegetation and the seed bank of the same slope we hypothesis that on fragmented habitats there are smaller populations with low seed amount and seed mass. This might lead to the disability to built up a well developed seed bank. To confirm this hypothesis further investigations will be done.

P1668. Soil seed bank in limestone grasslands on Miechowska Upland (southern Poland)

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The soil seed bank and vegetation were studied in two limestone grasslands (*Inuletum ensifoliae*, *Festuco-Brometea*), over a 2-year period. At both sites 4 permanent plots (25m² each) were established in patches representing different successional stages: initial grassland, well developed, overgrown by shrubs and a proximate oldfield colonized by xerothermic species. The soil samples were collected in summer and autumn each year and the seed bank size and composition were determined by two methods: greenhouse germination over a 6-month period and seed extraction by washing. The seed density varied from 950 seeds/m² in overgrown grassland to 12000 in oldfields (mainly *Hypericum perforatum*). Over 60 species were found in the seed bank of both sites. Among them 12 were present only in the seed bank. The species richness of soil seed bank was the highest in oldfields (30 species), the lowest in initial grassland (10). The viable seeds of xerothermic species e.g. *Inula ensifolia*, *Linum hirsutum*, *L. catharticum* were found in soil also in overgrown plots. This suggests that soil seed bank can play a certain role in the restoration of such grassland after shrub removal.

P1669. Prairie species from a temperate environment exhibit positive germination responses to smoke treatments

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Fire has been a natural disturbance in grassland ecosystems since the Holocene period. In the Midwestern USA, for example, fire determines, in part, the structure of the landscape as prairie, savanna or woodland. Prairies require a high fire frequency to reduce the encroachment of woodland species. In other fire-prone environments (Australian, South African and American Mediterranean ecosystems), seeds will only germinate in response to fire-related cues such as smoke. Without these cues, the seed may remain dormant. The effect of smoke on prairie seeds had not previously been investigated.

This study treated seed of prairie species with aerosol smoke and smoke water under glasshouse and field conditions to identify which species were stimulated by smoke. The results were mixed; some species experienced increased or decreased germination percentages while others were unaffected. The results of this study suggest that the positive effect of smoke on seed germination extends further than that of Mediterranean environments. Some species from temperate environments that are historically prone to fire produce seeds that germinate in response to smoke.

P1670. Berg's hypothesis revisited: Flower integration in populations of *Narcissus papyraceus* (Amaryllidaceae) with contrasted pollinators.

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It was postulated long time ago (Berg RL 1959, 1960) that species pollinated by specialist pollinators (the ones that exert more selective pressures, should present flower traits tightly correlated, compared to flowers pollinated by generalist pollinators (less selective pressures). Additionally, the first kind of species would show less variation, as result of stabilizing selection on these traits. However, the available studies support or reject the hypothesis, probably because of the lack of precise knowledge on the pollination biology of the species or adequate population sampling. Here, we report data on pollination biology, flower and vegetative trait correlation (the last one as control) of a winter flowering species, *Narcissus papyraceus*, which presents geographically separated populations, pollinated mainly by hoverflies or moths. Populations pollinated by moths tend to show highly correlated and integrated flower morphology compared to those pollinated by hoverflies, as Berg predicted.

P1671. Floral Morphology, Flower Longevity, and Nectar Secretion Patterns of the Holoparasitic Plant *Cytinus hypocistis* (L.) L. (Cytinaceae) Growing on Three Different Hosts

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Floral morphology, flower life span, and patterns of nectar production were examined in the monoecious *Cytinus hypocistis*, a root holoparasite of some species of Cistaceae. Variations of these features were analyzed in six populations on three hosts, two on *Cistus ladanifer*, two on *Cistus salviifolius*, and two on *Halimium halimifolium*. In all the populations the female flowers were bigger, opened earlier, and secreted a more concentrated nectar than the male flowers. Flower life span ranged from 4 to 9 days and, regardless of the host, in some populations female flowers lasted more than male flowers, whereas in other populations we encountered the opposite outcome. The amounts of nectar secreted in 24 hours ranged from 0.03 to 8.72 μ l, and nectar concentration varied from 12% to 50%. Nectar secretion differed among plants and populations, but it was the same among hosts. It was noticeable that, in spite of the huge variations, all the monitored flowers had some quantity of nectar at any hour of the day. Although this study is based only on some floral characteristics, our results do not allow us to suggest ecological races of *C. hypocistis* associated to each host.

P1672. Nocturnal anthesis of caper: Does time matter?

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Caper (*Capparis spinosa* L.) is a winter-deciduous, invasive plant; its flowers consisting of four green sepals, four white petals, numerous purplish stamens and a flexible style last for 12 hours, during anthesis that coincides the dry summer. Nocturnal, floral advertisement presents adaptations to prevent the warming effect of exposure to solar radiation and to avoid the evaporative cooling of floral tissues at the expense of water. Stomata on surfaces of sepals and of petals may be involved to the cooling of flower. Petals contain vacuolated parenchyma cells with large intercellular space. Filaments and style densely packed with small cells are related to cell wall material that provides strength. By blossoming released at sunset, the species avoids water expenditure, minimizes exposure of flowers to diurnal solar radiation and to elevated, damaging temperatures. Corollas grow close to the ground, where midday temperatures can exceed 40 °C. In turn, white corollas are exposed to visits of pollinators by remaining turgid overnight. This may be partially due to a decline in the solute potential of petals, via withdrawal of water from other floral tissues.

P1673. Nystinastic movements do not necessarily involve an increase in net pre-emergent reproductive success. The case of *Silene colorata* Poiré (Caryophyllaceae)

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Silene colorata Poiré (Caryophyllaceae) draws pollinator attention only by night by means of nystinastic movements but can be pollinated by both diurnal and nocturnal insects. We set an experiment to test the final influence of these temporally segregated pollinators and found that nocturnal fruit-set was higher than diurnal one but, additionally, that flowers exposed in daylight hours suffered damage due to *Bombus* activity; as this damage avoided a successful pollination, the found difference vanished once it was included as a covariate. Thus a higher fruit-set in individuals exposed by night was due to a lower flower damage and not to a higher pollination; anyway, as the number of visits by night was lower than by day, nocturnal pollination was more efficient. No difference among treatments was found for the seed-set, i.e. the number of ovules fecundated, once flowers had been pollinated, was the same. These data do not support the hypothesis that the flower display is an adaptation to attract pollinators increasing the fruit-set. On the other way round, by means of less conspicuous flowers, this species could avoid attracting predators when more abundant.

P1674. Butterfly pollinators select for short flowers in the carnation *Dianthus carthusianorum*

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Interactions with pollinators have resulted in a spectacular diversity of flowering plants. Pollinator-mediated selection has repeatedly been shown, but most quantitative studies have taken only floral or pollinator traits into consideration. We conducted field surveys and experiments, measuring variation in flower tube length of the butterfly-pollinated *D. carthusianorum* and pollen deposition of two pollinating butterfly species with varying proboscis lengths. We found that (1) seed set is pollinator limited in the field, a precondition for pollinator-mediated selection, (2) selection depends on the mechanical interaction between flowers and pollinating butterflies, and (3) both experimental butterfly pollinators select for shorter reproductive floral parts (anthers and stigma lobes) and hence for shorter flowers. This study shows not only how quantitative morphological traits of flowers and pollinators affect floral selection, but demonstrates also a process counteracting an evolutionary race between proboscis length and flower depth. These results may explain the rarity of very deep flowers.

P1675. How pollinators, seed predators and grazers influence selection on floral display of *Primula farinosa*.

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Floral display characters may influence the attractiveness to pollinators, but also the risk of damage by seed predators and grazers. We examined how biotic interactions influence selection on scape length of *Primula farinosa*. This perennial herb is dimorphic for scape length and produces either a regular or a very short scape. We studied four populations over two years on Öland in Sweden. At fruit maturation we measured fruit initiation, seed predation and proportion of grazed plants. To estimate the role of pollinators in the process of fruit initiation, we supplementally hand pollinated half of the study plants. Short-scaped plants of *P. farinosa* had in most cases lower fruit initiation than long-scaped plants and they were in general more pollen limited. However they were less susceptible to grazing and seed predators. Because of variation in the magnitude of these morph-specific differences, the relative fruit set of the two scape morphs varied significantly among populations. As intensive grazing influences the habitat, we also discuss how the scape-morph specific interactions with pollinators and fruit predators might be affected by vegetation height.

P1676. Floral evolution along altitudinal gradients in *Campanula rotundifolia* (Campanulaceae)

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We investigated patterns and mechanisms of floral evolution in the perennial *Campanula rotundifolia* (Campanulaceae) along altitudinal gradients in Norway. Flower size was explored in 16 populations at altitudes between 240 and 1100 m a.s.l. We

estimated phenotypic selection on four morphological characters through three fitness components in two of the populations. Flower length covaried positively with elevation. Plants in a high-elevation population (800 m a.s.l.) were shorter and produced fewer, but larger, flowers than in a low-elevation population (510 m a.s.l.). Seed set per capsule was higher in the high than the low population, but seed set per shoot did not differ. Selection on flower size was generally through seed set per capsule, while selection on plant size was through capsule number and did not differ in strength between the two populations. Pollinators probably caused the observed selection, although we do not know whether selection on flower size was generated by variation in the visitation rate or in pollination efficiency per visit. Selection mediated by pollinators may contribute to the evolution of larger flowers at higher elevations.

P1677. Assessment of genetic variability in some Iranian sweet oranges (*Citrus sinensis* [L.] Osbeck) and mandarins (*Citrus reticulata* Blanco) using SSR markers

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The genetic variability of 8 sweet oranges and 6 mandarins accessions was evaluated using simple sequence repeats (SSRs) analysis. In total, 52 putative alleles were detected using 7 primer pairs. The number of putative alleles per primer pair ranged from 3 to 10 with an average of 7.42. Polymorphic information content (PIC) value changed from 0.505 to 0.950. Microsatellite markers discriminated variation within mandarins, but low variation observed between sweet oranges.

A UPGMA phenetic tree was constructed and one main sweet orange group consisting of three sub-groups and four main mandarin groups were identified.

Results showed that; the majority of local sweet orange accessions probably have a narrow genetic base suggesting that the observed morphological polymorphism within the group must be associated with somatic mutations or they may be single clone with different names, which were not exactly detected by these markers. Studies with more microsatellite markers or other molecular markers may be more precise in determination of genetic variability among selected genotypes.

P1678. Molecular biological studies on *Digitalis lanata* Ehrh. populations

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Digitalis lanata Ehrh. populations were selected in the Mecsek, in a mountain of south Hungary, in the Great Hungarian Plain and a *Digitalis ferruginea* population in Italy. These plants were dried at room temperature and the DNA was isolated from the samples. The samples were prepared with several primers (OPN, OPO, OPP and OPW-primers), an F-buffer and a Taq-polymerase. The samples were tested and studied by RAPD-technique. The samples were developed on agarose gel. After development photos of the gels were taken at UV light. The bands were evaluated by comparison to the marker bands. In the DNA-patterns altogether 2-4 bands per plant were detected. The plant DNA-patterns contained uniform and unique bands equally. The unique bands of the populations refer to polymorph primers. On the basis of these results the plant populations could be classified into groups. This classification of genotype by RAPD can be related to the phenotype and the ecological habitat of the leafy foxglove populations.

P1679. Interspecific genetic diversity in European oak populations based on the fragment length analysis of a single beta-tubulin gene

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The genus *Quercus* is characterised by a high variability of traits and the tendency to hybridize without significant mating barrier. For the two highly intercrossing species *Quercus robur* L. and *Q. petraea* (Matt.) Liebl., diversity studies showed low interspecific genetic variation. In this study we report the isolation of a TUB homologue of *A. thaliana* from oak, which proved to be highly polymorph in natural populations. Two alleles were found to be selectively associated with either sessile or pedunculate oak.

Population differentiation based on allele frequencies of TUB was significant within nine out of 10 investigated continuous mixed oak stands. A correlation between specific alleles and differentiation was assumed by the above-average contribution of the two indicated alleles to the overall *F_{st}* value. Differences in the segregation of TUB were detected within a *Q. petraea* intraspecific and an interspecific cross attributed to the inheritance of the "petraea" specific allele. Hence, differences in gene flow detected in artificial hybrids as well as in natural oak populations could be explained by a hybridisation barrier between *Q. robur* and *Q. petraea*.

P1680. Analysis of genetic diversity of Iranian pistachio (*P. vera*) cultivars based on RAPD and morphological markers

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The Random Amplified Polymorphic DNA (RAPD) technique and morphological markers were employed for characterization and determination of genetic relationship in some of the major Iranian pistachio (*P. vera* L.) cultivars. Among 77 primers, only 15 primers revealed polymorphism among the cultivars. Based on Jaccard's similarity coefficient and UPGMA algorithm, cluster analysis, placed the pistachio genotypes in three main groups including; cultivated *P. vera*, wild *P. vera* and *P. mutica*. Cluster analysis results indicated that Gholamrezaei and Momtaz cultivars were identical. Wild *P. vera* named Sarakhs was closely classified with Italiaei cultivar. This strengthened the postulate which says the Iranian commercial pistachio cultivars were originated from sarakhs variety. Cluster analysis of 29 morphological quantitative traits and 17 qualitative ones showed concordance between grouping pattern of these data and those ones of RAPD.

Keywords: RAPD, Genetic diversity and pistachio

P1681. Genetic variation and phylogeographical patterns in *Alsophila podophylla* from southern China based on cpDNA *atpB-rbcL* sequence data

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CpDNA *atpB-rbcL* intergenic spacers of individuals of *Alsophila podophylla*, collected from eight populations distributed in Hainan and Guangdong Province, southern China, were sequenced. Sequences were assessed as evolutionarily neutral. Eight haplotypes were identified based on a statistical parsimony algorithm. A high level of haplotype diversity and a low nucleotide diversity were detected. Populations from Hainan shared common haplotypes with those from Guangdong. A network and a NJ tree constructed from haplotypes both suggested a close genetic relationship among populations distributed in Hainan and Guangdong. *F_{ST}*, *N_m*, AMOVA, and DNA divergence data consistently indicated that no geographical differentiation occurred at the interregional level. Geographic isolation has not yet resulted in population differentiations within the populations in Hainan and Guangdong. Phylogeographical patterns demonstrate a 'star-like' feature. The majority of haplotypes coalesced near the tip of the NJ tree. Moreover, a demographic signature of population expansion was also detected by mismatch distribution analysis.

P1682. Genetic diversity of two *Ephedra distachya* populations in the Alps

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The genus *Ephedra* occurs in Europe with nine species. In the Alps, *Ephedra distachya* s.l. (including *Ephedra helvetica*) is a rare species of the central alpine dry valleys such as in Wallis, Switzerland or the Durance Valley in Eastern France. During the Pleistocene with its great glaciers in the central Alps, *Ephedra distachya* probably survived in the unglaciated parts of the Provençal Alps. After this period, their migration routes, delimitation of infraspecific taxa, their taxonomic relevance and relationships between populations are still unknown. For example, in the literature the few occurrences in South Tyrol were assigned to two or three taxa on the basis of differences in the form of the micropyle of the female flower. This poster reports the initial findings of a molecular study of *Ephedra* populations in the Wallis,

Switzerland and Vinschgau, Italy. They reveal little genetic variation between the investigated populations and suggest that both populations belong to one taxon.

P1683. Molecular Variation in *Cordyceps sinensis* on the Tibetan Plateau

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Cordyceps sinensis is a fungus growing on larvae of moths and endemic on the Tibetan Plateau with patched distributions. It is one of the most valued herbs in the Traditional Chinese Medicine. DNA was extracted from stroma, sclerotium and living strain of fruitbodies collected over the Plateau, and 53 ITS sequences were obtained from 33 samples. Combined with the 118 sequences retrieved from the GenBank, excluding 83 of ambiguous locality, repeated submission or unreliable sequence readings, analysis of 88 sequences revealed no difference in sequences from stroma, sclerotium and living strain from the same individual, apart from an occasional 8 bp deletion. Thirty-one haplotypes were identified with pairwise distance ranging from 0.00205 to 0.02664. Two major clades were found in the phylogenetic tree, with samples from Yunnan in one and those from Sichuan, Qinghai and Xizang in the other. However, the molecular variation of ITS sequences within *C. sinensis* is much lower than that among species. It is concluded that diverse host species of *Hepialus*, over 30 reported, and vast geographic separation have not induced much variation in ITS sequences of *C. sinensis*.

P1684. Allozyme variation in five natural populations of *Juniperus sabina* L. in Spain

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J. sabina is a small dioecious, wind-pollinated shrub with usually more or less prostrate habit, scale-like leaves and fleshy cones. It occurs on mountain areas of Eurasia and shows notably fragmented distribution pattern. Nine loci were assayed applying starch gel electrophoresis for five parental (fleshy husks of cones) and four progeny populations (embryos). All enzyme systems proved to be polymorphic both to parental and descendent plants. Locus DIA appeared to be most monomorphic whereas locus SHDH was most variable. Population from the Betic Cordillera was most genetically diverse. A small differentiation among populations and large variation within populations were reflected by small value of GST. All groups of embryos are characterized by an excess of homozygotes relative to Hardy-Weinberg equilibrium. Excess of heterozygotes was observed in case of mature individuals and only population from Sierra de Albarracín showed not large surplus of homozygotes. Mean values of observed heterozygosity of parental populations keep level with other coniferous plants. Nei's genetic distance reflected geographical distribution of populations.

P1685. Genetic diversity of holm oak (*Quercus ilex* subsp. *rotundifolia* (Lam) O. Schwarz) in Portugal

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Q. ilex subsp. *rotundifolia* and *Q. suber*, keystone species of traditional Mediterranean silvi-agroecosystems, cover 2-2.5 million ha in the Iberian Peninsula and occupy critical areas in terms of soil and water resources. There has been a severe decline of these species during the last century, resulting from deforestation, unfavorable climatic factors, epidemics and overgrazing, which prevented regeneration. Long-term sustainability of these silvi-agroecosystems is further threatened by global warming. Knowledge of the distribution of genetic diversity is critical to the development of effective species conservation and utilization, and for the long-term sustainability of these ecosystems. In this study, regional genetic variation of holm oak was investigated using SSRs. Mean genetic diversity was high. A significant F_{ST} value and positive F_{IS} value indicate some differentiation among populations and only a low level of inbreeding. The results suggest high outcrossing rates, but also differentiation among populations and relatedness among trees of the same population resulting from common origin and adaptation.

P1686. Using RAPD and AFLP markers for molecular characterization of a *Theobroma* genus germplasm bank

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RAPD and AFLP markers were compared in terms of their discriminative capability and genetic relationship establishment among 65 *T. grandiflorum* and 16 *T. bicolor* materials from an *ex situ* *Theobroma* germplasm bank (Guaviare, Colombia). Similarity and principal component analyses were done for 117 RAPD and 375 AFLP bands. A low congruence in the results for each marker was determined from differences in dendrograms and correlations of similarity values. The analysis of AFLP markers showed a higher correspondence than RAPD with the agronomic groups of some *T. grandiflorum* materials and ecotype classification of *T. bicolor* materials. When the analysis implies materials from both species, the expected heterozygosity values were highest for RAPD (0.30) than AFLP markers (0.25), this value were not significantly different for the independent analysis of species. The marker index values indicate the highest overall utility of AFLP markers system for materials discrimination. This is particularly important for *T. bicolor* materials due the high similarity values estimative (0.9 - 1.0) found by RAPD markers, as a consequence of its low polymorphic markers fraction.

P1687. Inventory and economical value of genotypes from naturally widespread population of black mulberry (*Morus nigra* L.) in Slovakia

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Monitoring and inventory of widespread genotypes from the black mulberry population (*Morus nigra* L.) in Slovakia was detected by GIS technology. Most of the genotypes exceeded the age of 200 years. By experimental study of 480 genotypes were determined traits variability on the level of trees, leaves, flowers, fruits and seeds.

Leaves length was in the range 34 - 217 mm, width 36 - 193 mm and the stem length 4 - 46 mm. In the fruit level were determined weight in the range from 34 to 127 g, length 8.1 - 34.4 mm, width 5.3 - 25.2. There was evaluated juice yield (75%), content of vitamin C (11.09 - 14.95 mg/100g), pigments, sugars and organic acids also. Experimental data are utilized for developing the descriptor list. Basic data about genotypes with image documentation are processed in information system GENOTYPDATA *Morus nigra* L. using ICT.

P1688. Assessing the genetic diversity of *Goetzea elegans*, an endangered Caribbean Tree, based on AFLP data

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Recent advances in molecular technology have opened a new chapter in species conservation efforts, as well as in population biology. One of such techniques is the amplification fragment length polymorphism (AFLP), based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. We investigated levels of genetic diversity within and among the last remaining populations of *Goetzea elegans* Wydler (Solanaceae), a highly endangered tree species endemic to Puerto Rico. We used an AFLP protocol based on infrared detection of AFLP fragments and the number of polymorphic fragments obtained were visualized and scored using LI-COR automated DNA analyzer and the SAGAmx software. Current results indicate the presence of both, intra and inter-population polymorphism that allows developing a preliminary assessment on the current genetic diversity of the species. The data obtained in this work have important implications for conservation and future breeding programs of this and other rare plants of the Caribbean.

P1689. Development of microsatellite (SSR) markers in *Phaseolus radiatus* (mungbean) and their application on diversity assessment

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Microsatellites (SSR) are the most useful genetic markers today. Because of their high polymorphism and relative ease of scoring, the demand for these markers is increased rapidly. We have developed a simple, fast and efficient procedure for generating highly SSR-enriched library from genomic DNA of mungbean (*Phaseolus radiatus*). The successful construction of SSR-enriched library relies mainly upon high ratio of SSR containing clones, low redundancy and sufficient number of flanking sequences for primer designing. The library constructed by our new procedure contains up to 80% SSR positive clones. Direct sequencing of randomly selected clones for primer designing facilitated to the fast development of 87 SSR primers. Genetic profiling of 80 selective accessions of mungbean with 24 developed SSR primers has been successfully conducted to date. The efficiency of SSR development and diversity of mungbean collection using SSR markers will be discussed based on germplasm management and genetic diversity.

P1690. Sex determination and sex chromosome evolution in *Silene dioecious* species

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Most flowering plants are hermaphrodite. Dioecious species, having separate male and female individuals represent only ~4% of angiosperm species. The *Silene* genus (Caryophyllaceae) contains a clade of dioecious species (including *S. latifolia*, *S. dioica* and *S. diclinis*) whose sex determination is based on a XY sex chromosome system with a male-determining Y. This genus is thus well-suited as a model for studying plant sex chromosome evolution.

Sex chromosomes are thought to evolve from an ancestral pair of autosome, between which recombination in the sex determining region has ceased. Sex chromosome evolution, and the causes of recombination arrest and Y degeneration, have been studied in animals. Thanks to 4 genes isolated in sex chromosome of *S. latifolia*, it is now possible to do similar studies in the plant kingdom. We first demonstrated that these 4 genes have an orthologs on sex chromosomes in *S. dioica* and *S. diclinis*, suggesting a common and recent, origin of sex chromosomes. We also mapped the 4 genes on the X chromosome and compared divergence of gene pairs between the X and Y copies. We discuss the results of this analysis and compare them with animal systems.

P1691. An evaluation of microsatellite markers in identification of zygotic seedlings in *Citrus* breeding programs

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Microsatellites or SSRs (simple sequence repeat) have been employed as reliable markers in the plants genome analysis. In citrus breeding and genetic programs, it is very important to distinguish zygotic and nucellar seedlings in order to eliminate unwanted genotypes. This study details the properties of four example SSRs markers that were isolated from "Lisbon" lemon (*Citrus limon*) using the bead-enrichment method for distinguishing zygotic from nucellar seedlings of a cross between "Page" mandarin (as female) and some *Citrus* cultivars (as male). Four SSRs loci including *AMB2*, *AMB5*, *AMB8* and *AMB10* were tested for amplification of target loci both within the parents and progeny. The results indicate that more than 90% of target loci in each experienced genome can be amplified by tested primers, so it shows that SSRs flanking primers to be conserved in every genome tested. In addition, SSR analysis for the identification of hybrids derived from the cross of polyembryonic citrus cultivars was efficient and the genotype of every plant could be unmistakably assessed.

P1692. Study of Sexual Reproductive system in Marigold (*Calendula officinalis*) and evaluating of Cross-pollination

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Morphological study of *Calendula officinalis* can be important for increasing of essential oil and other components. In order to investigate of sexual structure and reproductive system of marigold an experiment was conducted during 2001 growing season at Hamadan, Iran. In this study, we evaluated sexual structure of flowers, types of florets, morphological structure of seed and percent of cross-pollination. Resulted floret included two types. The outer-floret names ligular capitule that it has sterile female organs. The inner floret names tubular capitule that it has both sterile female and hermaphrodite organs. Stigma is two branches shapes and male organs have three continuous stament. Results showed that the percent of cross-pollination at 95% of confidence interval is 94.73 ± 1.13 .

P1693. GENERATIVE CELL SPECIFIC 1 is essential for angiosperm fertilization

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The double fertilization process in angiosperms is based on delivery of a pair of sperm cells by pollen tube elongation toward each embryo sac enclosing egg and central cells. Several studies have revealed gametophyte interaction mechanisms, however, nothing is known about the gamete interaction. In this study, we identified a novel protein, designated GCS1 (GENERATIVE CELL SPECIFIC 1), using generative cells isolated from lily (*Lilium longiflorum*) pollen. GCS1 possesses a C-terminus transmembrane domain, and immunological approaches showed that it is accumulated during the late gametogenesis and localized on the plasma membrane. Arabidopsis *gcs1* mutant showed the serious male sterility. This finding would be the first report of the gamete interaction factor essential for the successful angiosperm fertilization. Noteworthily, GCS1 homologues were identified in algae, slime mold and parasites, and the gamete specific expression was revealed in such non-angiosperm species. Our findings might give some important clues to understand the evolution in the gamete interaction.

P1694. Pollination ecology and visitors diversity of *Monotagma plurispicatum* (Körn.) K. Schum. (Marantaceae) in a tropical Atlantic rainforest remnant, Northeastern Brazil

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A case history of the herbaceous *Monotagma plurispicatum* was realized from February to April 2005 in a natural population of two different border sites of an Atlantic rainforest remnant with 3500ha in extent (8° 30' S, 35° 50' W), in Alagoas state, northeastern Brazil. The study was focused on floral biology, pollination mechanism, reproductive system and nectar production of the species. With an entomophilous syndrom, *Monotagma* presents axilar inflorescence with hermaphrodite flowers of white color and diurnal anthesis and offers nectar as unique floral resource. Characterized by secondary pollen presentation, the flowers show a highly precise and irreversible explosive mechanism to discharge the pollen. In spite of presenting a considerable range of visitors, only those who are able to release this mechanism can be considered as effective pollinators, such as two species of Euglossinae bees (genus *Euglossa*) and one hummingbird (*Phaethornis ruber* - Trochilinae). Differences between the two studied areas concerning richness and frequency of the visitors indicates that forest fragmentation could have an impact on the pollination ecology of the species.

P1695. Carpenter Bee Pollination In Two *Crotalaria* Species (Fabaceae)

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Crotalaria laburnifolia L. and *C. verrucosa* L. are perennial shrubs with robust growth during rainy season. They flower year-long with concentrated flowering during August-September. The terminal racemes produce yellow flowers in *C. laburnifolia* and blue flowers in *C. verrucosa*. In both, the flowers are characteristically papilionaceous representing flag-shaped blossoms with pump type pollen presentation mechanism. The flowers are nectariferous, open from noon to evening time with a maximum percentage at 1500-1600 h and were visited regularly by carpenter bees (*Xylocopa latipes* and *X. pubescens*) during the period of anthesis

and occasionally by digger bees and leafcutter bees at any time of the day for collecting nectar. The flowers were frequented more by juvenile carpenter bees than their adult ones and the probing behaviour resulted in sternotribic pollination. The bees showed inter-plant foraging activity in quest of more nectar. As both *Crotalaria* species are self-compatible, the nectar-feeding activity of bees, especially carpenter bees has been considered to be effecting both self- and cross-pollination.

P1696. Estimating selection on floral traits through male fertility in a large natural population of *Mimulus guttatus* by using the neighborhood model

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Most methods for studying selection through male fitness using genetic markers require that all potential males in the population are sampled. We used the neighborhood model, which also considers outcrossing with non-sampled background males, to estimate paternal selection gradients on floral traits in a large natural population of *Mimulus guttatus*. Multivariate analyses indicated that male mating success declined with distance from and depended on the direction to the mother plants, and that there is directional selection for plants with fewer open flowers which have more elongated corollas, smaller anthers, a smaller anther-stigma separation, more red dots on the corolla and a larger fluctuating asymmetry therein. However, for most of these traits there was also stabilizing selection for intermediate optima. The large number of significant selection gradients in our study shows that even in relatively large natural populations where not all males can be sampled, it is possible to detect significant paternal selection gradients when the analyses are based on the neighborhood model.

P1697. Fruit set of the endemic *Barlia metlesicsiana* (Orchidaceae) on Tenerife Island

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The distribution of the orchid species *Barlia metlesicsiana* W.Teschner is restricted to the Canary Island Tenerife. Only two populations are reported from the western part of the island: Santiago del Teide and Chio/ Guia de Isora.

Both populations are characterised by a patchy population structure with several subpopulations and individuals growing solitary, and therefore, plants are difficult to reach for pollinators. We analysed 230 individuals of four subpopulations representing both known populations. Overall, one third of the analysed plants flowered in 2004. Portions of flowering individuals ranges from 19.0-56.3% in four subpopulations with an average of 26% of all flowers (n = 1805) producing matured capsules as a measure of pollination success. Additionally, we recorded plant height, leaf characteristics, and flower number per plant. Correlations reveal no significant relationship between plant height or flower number ("floral display") and individual proportions of fruit set indicating almost random pollination success. As effective pollinator the bumble-bee *Bombus canariensis* Pérez was observed.

P1698. Sex ratio and differences between male and female specimens in *Taxus baccata*

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Sex ratio in 4 natural and 1 spontaneous semi-natural populations of *Taxus baccata* were verified in Poland and Ukraine during 2003. The aim of the study was to verify the hypotheses, that: 1) sex ratio depends on a) the average age of population, b) environmental conditions, 2) dimensions of trees and needle characters of male and female individuals are different. The sex was verified on the plots within of all 5 populations on the basis of presence of micro- and/or macrostribili. Sex ratio, counted as quotient of female and male specimen numbers, varied from 0.49 in the oldest to 1.12 in the medium aged population. Sex ratio correlated statistically significantly with the average yearly precipitation. The male individuals were higher and thicker than female ones, but the female individuals have longer needles than male. It was concluded, that female individuals, have probably higher reproductive costs and can to lose the competition with the male ones.

P1699. Genetic and molecular analysis of trichome density using naturally occurring variation among *Arabidopsis thaliana* accessions

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Arabidopsis thaliana trichomes are single epidermal cells present on leaves, stems, petioles and sepals. The morphological and mechanical features of trichomes can influence many aspects of plant physiology and ecology and thus may be of selective importance.

Genetic studies identified a well defined set of genes including (MYB related and bHLH-like) transcription factors and a WD40 repeat protein that regulate trichome initiation. The current model of de novo trichome patterning involves the action of activators and inhibitors, which account for the regular spacing through local self-activation and lateral inhibition. Furthermore, across *A. thaliana* accessions trichome density on leaves shows a wide variation. We exploited the natural occurring variation for mapping novel modulators of trichome density in a QTL analysis. We chose a F2 mating design, based on a cross between two extreme *A. thaliana* accessions, with low and a high trichome density. Molecular and population genetic analyses of candidate loci will contribute to the understanding of trichome patterning and of adaptive processes acting on this trait.

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P1700. Life history plasticity to irradiance during seedling establishment in *Picea omorika*

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The adaptive value of life history plasticity to irradiance has been tested in *Picea omorika* seedlings from a synthetic population comprising 21 maternal families from six natural populations. Phenotypic variation induced by two distinct light intensities was explored for three juvenile traits: days from germination to cotyledon opening (DGTOC), days from cotyledon opening to epicotyl appearance (DCTOE), and epicotyl length at six weeks (EPL6). Ambient light conditions affected significantly DCTOE and EPL6, but not DGTOC. Phenotypic selection analysis revealed that canalization of DGTOC was favoured by selection, but entailed a fitness cost under both light levels. The plasticity of DCTOE to irradiance was diagnosed as maladaptive. The existence of heritable variation for plasticity was revealed for all of the traits studied. However, since DGTOC and DCTOE were involved in a genetic trade-off, these traits would never reach their optimal values across radiation environments.

P1701. Adaptive plastic responses to an irradiance gradient in the *Iris pumila* plants from exposed and shaded habitats

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Plasticity of *Iris pumila* leaf traits (three morphological and five anatomical) to irradiance was explored under growth-room conditions. Siblings from 28 full-sib families from an open and a shaded population responded similarly to changes in light availability: morphological traits gradually increased, while anatomical traits decreased with light reduction. Comparisons of the induced phenotypes with those favored by selection at each light level revealed that a larger leaf size and an increased SLA were functionally beneficial on light-deprived conditions. A medium stomatal density was also adaptive at high light in the open population. Plasticity costs were detected for two anatomical traits at low irradiance in the open population, as well as one morphological trait at medium light, and one anatomical trait under high irradiance in the shaded population. The cost of canalization was recognized for two anatomical traits at high and low light, and one morphological at low irradiance.

P1702. Phenotypic plasticity and genetic differentiation of *Diplotaxis eruroides* (Brassicaceae) populations in Sicily

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Diplotaxis eruroides is a wide-spread species in the mediterranean area. Particularly, in arable fields it occurs with at least two flowering generations per year (winter-annual, summer-annual). Different phenotypes are found according to the different environmental conditions of humid winters and arid summers. The present study aims to quantify the phenotypic differences among the generations and to estimate the extent of their genetic differentiation. The soil seed bank is included to illuminate the genetic diversity and composition of the belowground subpopulations in relation to the genetic constitution of the aboveground subpopulations. In Sicily seven populations were sampled (30 individuals / generation, soil samples). Phenotypic variation is assessed by morphological traits as height, branching, fruit production, leaf characteristics and life history traits like speed of development (common garden experiment). Genetic variation is analysed using AFLP technique. Correlation of phenotypic and genetic variation will indicate, to what extent phenotypic differences among generations are due to plasticity or ecotypic differentiation.

P1703. Individual variability of mineral content in relation to plant size in experimental populations of two herbaceous species

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Populations of white mustard (*Sinapis alba*) and small balsam (*Impatiens parviflora*) were grown in glasshouse conditions, each species at two population densities. Measurements of individual size taken at harvest included: stem height and diameter, aboveground dry mass, leaf area, crown projection area etc. Concentrations of N, P, K, Ca, Mg, Na, Mn, Fe and Zn in leaves and stems were determined. Patterns of size-concentration relationships differed both across species and densities. The percentage of variation in element concentration explained by the set of size measurements ranged from about 0% (N and P in low-density *Impatiens*) to 39% (N in *Sinapis* and high-density *Impatiens*), to 64% (Zn in high-density *Impatiens*). Direction of some mass-concentration relationships depended on density (e.g. mass-K correlation: negative in low-density, and positive in high-density *Impatiens*). Also, various measures of size related differently to particular concentrations (e.g. for Zn in high-density *Impatiens*: strong positive partial correlation with mass and negative with plant height) Detailed results are presented in relation to plant size allometry.

P1704. MAM Variation Determines Glucosinolate Profiles in *Arabidopsis lyrata* ssp. *petraea*.

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Glucosinolates are chemical defenses against insect herbivores and are present in the Capparales. Methylthioalkylmalate synthase (MAM) is required for side chain elongation in Met-derived glucosinolate biosynthesis in *Arabidopsis thaliana*. MAM variation and its evolutionary dynamics in natural populations are unknown, so we investigated whether MAM variation explains glucosinolate variation in a natural population of *A. lyrata* ssp. *petraea* in Central Europe. Using microsatellite markers to distinguish MAM alleles, we crossed individuals within the natural population, genotyped the progeny and determined that the MAM alleles from 6 of 16 crosses associate with particular glucosinolate profiles. Some alleles associate with longer chain glucosinolates while other alleles associate with shorter chain glucosinolates. Therefore there are variable MAM alleles within a population determining glucosinolate content. This provides a useful system to study the evolution of a resistance phenotypic trait since the underlying genetic variation that causes the phenotypic variation is known.

P1705. Evolution of plant defenses in *Arabidopsis lyrata* within the context of a natural herbivore community

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Herbivory can have significant impact on plant fitness, and plant defenses evolve in response to herbivore-mediated selection. In the Brassicaceae, both trichomes and plant secondary compounds

such as glucosinolates function as defenses against herbivores, and respond to artificial selection in model and crop species. However, we know little about the evolutionary dynamics of the integrated defense phenotype in natural populations. For one natural population of *Arabidopsis lyrata*, an out-crossing perennial herb closely related to *A. thaliana*, we quantified the ecological role of herbivory in the field, characterized defense-related phenotypes, and assessed the genetic basis of defense. Herbivory during three years of field observations was imposed by a diverse and varying suite of insects and mollusks. Only the abundance of Lepidopteran and Heteropteran larvae was significantly positively correlated with tissue loss and negatively correlated with flowering in *A. lyrata*. Using specialist and generalist Lepidopteran larvae in bioassays of plant resistance in the laboratory, we found a genetic basis in resistance to multiple herbivores. In a quantitative genetic study sampling widely from the above population, we found significant heritability for trichome density and glucosinolate composition. Whereas resistance to *Trichoplusia ni* (a generalist) was significantly correlated with glucosinolate concentration, resistance to *Plutella xylostella* (a specialist) was correlated with trichomes density. Thus, significant genetic variation for different mechanisms of defense are segregating within this single population of *A. lyrata*. Genetic correlations among alternative defenses are weak, and do not appear to constrain the independent evolution different components of the complex plant defense phenotype in response to a multi-species herbivore community.

P1706. Temporal abundance patterns of the desert annual plant *Allionia incarnata* related to rainfall and temperature, and effects of predation by the harvester ant *Pogonomyrmex rugosus*.

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Desert annual plants must adjust their developmental patterns to short and unpredictable rain events. Also, seeds are prone to be gathered by consumers and dispersers, controlling their distribution and abundance patterns. In Mexican deserts, *Allionia incarnata* is one of the first annual plant to appear in the rainy season and is a main resource for harvester ants. We describe abundance patterns of *A. incarnata* during five years in three permanent sites at the semiarid Zapotitlán Valley, México. In one of these sites, we explored the impact of the harvester ant *Pogonomyrmex rugosus* on the dynamics of *A. incarnata*. Using Generalized Linear Models we adjusted abundance response surfaces related to temperature and rainfall. It was found in ample temperature (20 - 30°C) and rainfall (20 - 80 mm) ranges with differences related to soil water retention capacities. We found a 9 % of *A. incarnata* in the soil seed bank, and a 23.4% of predated seeds by *P. rugosus*. This highlights the relevance of seed predation as a controlling factor in the dynamics of desert annual plants. Finally, we discuss the adjusting of life-history traits of this species to a bet hedging strategy.

P1707. Biology, ecology and invasion characteristics of *Campylopus introflexus* in the Czech Republic

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The poster summarizes project aims and preliminary results of a study on the moss *Campylopus introflexus* (Dicranaceae) in Central Europe. *C. introflexus* is one of the most strongly invasive species of bryophytes in Europe. The species appears to be native in the Southern Hemisphere. In Europe, it was collected for the first time in the British Isles in 1941. The moss has expanded eastward and the first collection in the Czech Republic is dated 1988. New localities have been recorded with growing frequency. Today, 23 localities are known in the Czech Republic, but a thorough survey of this critical moss does not exist.

The author's research is based on monitoring phytogeography (east boundary of actual spread), life history (reproduction, competitive abilities, etc.), and ecology (microclimatic characteristics, substrate requirements, etc.). Genetic patterns in the Czech populations are being studied by molecular methods (isozymes, RAPD). In vitro cultivation and cryopreservation of samples is also part of the methodology.

P1708. Genetic diversity of *Sphagnum fimbriatum* in the northern and southern hemispheres

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Sphagnum fimbriatum (Sphagnopsida, Bryophyta) is a bipolar species that occurs in the northern and southern temperate zones (Eurasia, North America, South America, New Zealand). In some areas of Central Europe *S. fimbriatum* is rare. In Switzerland it is included in the red list of endangered bryophytes.

The aim of this study is to test the genetic variability within and among populations in order to assess whether:

- There is a positive correlation between population size and genetic variability,
- the current distribution in Switzerland is a consequence of fragmentation of populations,
- the world-wide differences in abundance correlate with differences in genetic diversity.

DNA from twenty-two European populations was amplified with RAPD markers and the data analysed with hierarchical analysis of molecular variance. The genetic distances/similarities between pairs of populations were calculated.

The higher partition of the variation is found within populations. That might be due to large effective population sizes, or multiple colonization events.

Additionally seven Argentine populations were analysed and compared with the European ones.

P1709. Genetic diversity of *Sphagnum subsecundum* agg. populations in Portugal

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Over the past 20 years, studies have revealed levels of genetic variation in bryophytes that are similar to those found in vascular plants. This has led many to question the traditional view of bryophyte evolution, which holds that these organisms have a low evolutionary rate. The morphological similarities through time and space are partly explained by the presence of physiological and biochemical, rather than morphological, evolution (creating sibling species) and convergent evolution.

The *Sphagnum subsecundum* agg is a species complex taxonomic challenging as the morphological traits used to distinguish the species, in some areas of its European distribution, overlap and many plants present intermediate forms. Therefore population studies will allow understand more about its dynamics and clarify the taxonomic relations. In Portugal, these taxa are the most abundant sphagna, but reproduce more mainly through gametophore fragmentation; rarely it's possible to observe capsules. The main aim of this work is to compare genetic diversity patterns using ISSR of different populations.

P1710. Differences in the diaspore bank of bryophytes between two grassland types: the role of the life-strategies

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Diaspore bank of bryophytes in open and closed grasslands on dolomite rock has been investigated by means of seasonally collected soil samples. These habitats differ in vegetation structure and microclimate. The total cover of bryophytes at the surface and in the soil samples was complementary: closed sites, dominated by perennials, had greater cover values at the surface (lack of long-lived propagules), while colonist-dominated open sites showed higher cover in the soil samples (abundant stored, long-lived propagules, potential dormancy). However, cover values in both samples types were greatly influenced by the sampling date, since some species had different abundances depending on habitat type and/or sampling date. The number of species in soil samples of closed sites with more even conditions was slightly higher, which can be explained by the additional presence of perennials. Species absent or rare at both sites were detected in soil samples, however some frequently and abundantly sporulating species were absent from the diaspore bank. Differences in patterns detected at the two sites can mostly be explained by their different life-strategy spectrum.

P1711. Darwinian selection on a selfing locus

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The evolutionary transition from outcrossing to selfing is one of the most prevalent trends in flowering plants. In 1876, Charles Darwin proposed the reproductive assurance model to explain it, suggesting that selfing can be evolutionarily advantageous when pollinators or mates are scarce in spite of inbreeding depression. It was shown that a model plant *Arabidopsis thaliana* became a selfer by the loss of self-incompatibility genes *SRK* and *SCR*. The *pseudoSCR1* gene in 21 *A. thaliana* accessions has low levels of nucleotide diversity. This indicates that positive directional selection has driven the evolutionary fixation of the pseudogene allele of *SCR*. Coalescent simulation showed that the event most probably occurred at the range expansion ~17,000 years ago owing to the glacial retreats. These results support Darwin's model, since rapid expansion would be accompanied by scarcities of mates and thus selfing plants would have a selective advantage during long-distance dispersals.

P1712. An investigation of the inheritance procedure and estimating the number of controlling genes of some traits related to grain yield in barley (*Hordeum vulgare*) Afzal/Radical cross

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In order to study inheritance of traits related to grain yield in barley, F₁, F₂ and F₃ generations were made from Afzal/Radical cross. Parents along with F₁, F₂ and F₃ generations were planted in a randomized complete block, design with three replications. Head weight, head length, number of heads, number of spikelets per spike, awn length, hundred grain weight, grains per head and straw yield per head in different generations were recorded. Analysis of variance indicated that mean squares of generations were statistically significant for all the traits. Then, generation mean analysis was performed for all the traits. The results showed that additive, dominance and epistasis effects were effective for all the traits except awn length. Recently trait was controlled with only additive and dominance effects. Also, results indicated that dominance variance was the most important for inheritance of all the traits. Average broadsense heritabilities were between 69-89% for all the traits. The number of genes for the traits were estimated to be between one and five.

P1713. Development of the microalgae communities in the phytotelmata of *Sarracenia purpurea* L.

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The phytotelmata of *Sarracenia purpurea* are colonized by a variety of organisms including bacteria, microalgae, protozoa, and invertebrates. However, the microbial food web and the role of microalgae in pitcher plants are still poorly understood. The aim of this study was to determine the structure and the succession of the algal communities in an allochthonous population of *S. purpurea* in a fen (Saxonia, Germany) within one year. More than 100 taxa of microalgae were found in the phytotelmata representing up to 97 % of the total living biomass. Within one plant, different phytotelmata harboured different algae communities. Approximately 50,000 algae per ml water fluid were present in young phytotelmata, dominated by Chrysophyceae, whereas 200,000 algae per ml water fluid were present in one year old phytotelmata, mostly dominated by Chlorophyceae. In contrast, Bacillariophyceae dominated in the fen. The colonization pattern in exposed sterile artificial pitchers were similar to those observed in the phytotelmata. Thus, the colonization of the pitchers seemed to be randomly and not affected by plant - phytotelm community interactions.

P1714. Comparison of molecular phylogenetic reconstruction of different loci in Nepenthaceae

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The Malay Archipelago is considered as a center of secondary diversity of Nepenthaceae, the pitcher plants of the Old World tropics. Recent analysis of the cpDNA trnK intron supports this hypothesis showing all species as neighbor group to the isolated species from Sri Lanka, Seychelles and Madagascar. Accordingly, an origin of recent Nepenthaceae in the Indian Subcontinent is indicated.

In the course of our study, we identified a translocated pseudogene copy of the trnK gene in the Nepenthaceae which we assume to be located in the mitochondria. In addition a nuclear single copy gene was analyzed. Comparison between the three datasets allows new insights concerning the evolution of Nepenthaceae. Obviously recent Nepenthaceae underwent a bottleneck and inconsistencies in the phylogenetic reconstruction of the data sets indicate that the evolution of the group was influenced by hybridization and introgressions. One monophyly of species from Borneo and Sulawesi shows a high distance to the rest of the taxa using non cpDNA data sets. This indicates that the cpDNA phylogeny could be misleading and an origin of recent Nepenthaceae on the Sunda Shelf is feasible.

P1715. Pedological and microclimatic requirements of *Drosophyllum lusitanicum*

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Unlike the majority of carnivorous plants, *Drosophyllum lusitanicum* colonizes hot and arid sites in the Western Mediterranean. Soil requirements and the enduring of severe drought stress in summer are controversially discussed in literature.

We compare soil composition, microclimate, trapping activity and further physiological parameters of plants from natural sites in different developmental stages. *Drosophyllum* roots only in acid soil but never in the calcareous bedrock, contradictory reports are caused by inaccurate sampling. It tolerates very low concentrations of all macronutrients.

The macro- and microclimate are very harsh during the day; wind is the only factor significantly decreased by the plant population. In summer, water neither from rainfalls nor from soil is available. Nonetheless plants are green, produce trapping mucilage and proteases. We show that water supply comes from the daily dew and is taken in by cuticular pores on the leaves. *Drosophyllum* is vivid and fruits in all stages of succession, but germination was only observed on pioneer sites. Preservation of open and oligotrophic sites is essential for the survival of this species.

P1716. Phosphatase Activity in Glandular Structures of Carnivorous Plant Traps

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Phosphatase activity was studied in situ in glandular hairs and glands of carnivorous plants, especially in the *Utricularia* genus, as well as in species of other carnivorous genera. For the visualization of the phosphatase activity the ELF (Enzyme Labelled Fluorescence) - 97 phosphatase substrate was used. The phosphatase activity was found in internal trap hairs of 19 *Utricularia* species from 11 sections, in various internal hairs of *Genlisea* traps (both members of the subgenus *Tayloria* and *Genlisea*), both stalked and sessile hairs of *Pinguicula*, sessile hairs of *Byblis linifera*, emergences of *Drosera*, and in glands of *Cephalotus*, *Brocchinia reducta* and *Nepenthes*. Taking into account intensive and commonly found activity of the phosphatase in glandular structures of carnivorous plant traps in these taxa, digested animals are important source of phosphorus for these plants.

P1717. Microflora in the Traps of Pitcher Plants

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Carnivorous pitcher plants supplement their nutrition by trapping and digesting insects in fluid filled traps. It is a matter of debate if digestion is performed by the plant or by bacteria. Interestingly, the traps are not only killing animals, but host diverse communities of animals and microorganisms.

Whereas many studies on trap bound arthropods exist, only little is known about protists and microorganisms in the digestive fluid. We

observe microorganisms in the fluid of three genera of pitcher plants (*Nepenthes*, *Cephalotus* and *Sarracenia*) and study the physiological properties of 44 strands of bacteria and fungi.

We find significant differences in the microflora of these three genera (*Nepenthes*: bacteria, yeasts, filamentous fungi; *Sarracenia*: bacteria, yeasts, protozoans; *Cephalotus*: bacteria, filamentous fungi, algae, moss protonemata). Concerning digestion, we find proteolytic activity in 6 strands of bacteria, 4 acidify the pitcher fluid, 3 degrade sulfur.

Our results show that digestion is significantly supported by microbial action. Furthermore, pitchers are a complex microecosystem for bacteria, fungi, protozoans and algae.

P1718. The mucilage of *Drosera*, a carnivorous plant, habitat or battlefield for bacteria?

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Introduction: Carnivorous plants have developed unique strategies to compensate for limited nutrient availability in their habitats by capturing small animals. *Drosera*, a carnivorous plant, produces and secretes mucilage containing digestive enzymes to break down captured prey. It is still unknown if microorganisms contribute to this digestion process or if the degradation of prey is done by the plant alone.

Material and Method: We combined microscopical and molecular techniques like *Fluorescence In Situ Hybridisation*, PCR-amplification of 16S rRNA genes and *Field Emission Scanning Electron Microscopy* to detect and identify bacteria on the tentacles and in the mucilage of different *Drosera* species.

Results: The number of bacteria in the mucilage and on the epidermal leave surfaces of *Drosera* was very low. The amount and identity of the detected bacteria did not correlate with vegetation period or *Drosera* species. After the addition of bacteria into the mucilage disintegration of bacterial cell membrane within a few minutes, followed by cell lyses, was observed. Therefore we proposed that these bacteria were airborne contaminants rather than symbionts.

P1719. Pharmaceutical study of *Aldrovanda vesiculosa* trap movement

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Successive phases of *A. vesiculosa* trap lobe movement were studied. Stimulation by bristle (BS) or feeding with boiled egg-white (FS) was used to induce trap movement while plants were incubated in chemical solutions. After incubation in 0.01 mM LaCl₂ or 0.1 mM EGTA shutting, narrowing and opening of the trap were inhibited both after BS and FS that indicated Ca²⁺ role in trap lobes movement. Incubation in 0.05 mM LiCl for more than 3 hr prevented shutting and opening after BS that may indicate inositol-3-phosphatase involvement in those processes. Open traps closed spontaneously but shut traps half-opened after 30 min incubation in 0.01 mM HgCl₂. Pre-incubation in 5 mM mercaptoethanol prevented spontaneous shutting in HgCl₂ solution may propose involvement of Hg-sensitive aquaporins in trap shutting. 0.01 mM monensin prevented opening of traps after FS. It didn't affect shutting and opening after BS either narrowing after FS. Thus only opening after FS could be decided to result from cell growth. Study of *A. vesiculosa* trap lobe cell ultrastructure confirmed stated assumption.

P1720. Morphology and nrITS phylogeny of the genus *Pinguicula* (Lentibulariaceae), with special attention to embryo evolution

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The family Lentibulariaceae is characterised by great variability of embryo structure, including cotyledon number. In this respect, the family is probably unique among other families of Lamiales. The genus *Pinguicula* which comprises about 80 species is especially interesting due to co-existence of species with monocotyledoneous and dicotyledoneous embryos (Haccius & Hartle-Baude 1957). To elucidate putative evolutionary history of embryo, we have analysed nrITS sequences in 31 *Pinguicula* species from Old and New World. Besides, we have studied seed (including embryo)

morphology and anatomy in 22 species. Our molecular phylogenetic data are largely congruent with data on the same DNA region produced by Shimai & Kondo (2004), although they studied only 21 species. We suggest that cotyledon number was quite unstable in the evolution of *Pinguicula*. Some features of seed coat structure and flower morphology are much more congruent to nrITS phylogenetic data than cotyledon number.

P1721. Complex organisation of the waxy zone in pitchers of *Nepenthes alata* Blanco (Nepenthaceae)

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The waxy zone inside pitchers in most *Nepenthes* species is covered with a thick layer of epicuticular wax and was reported to serve mainly for animal trapping and retention. In *N. alata*, two layers of waxes are distinguished. These layers differ in their structure, chemical composition and mechanical properties, and they decrease the attachment of insects in different ways. The lower layer is composed of highly interconnected irregular platelets, whereas the upper one consists of densely arranged separate membranous platelets bearing a pedicel-like "foot". These morphological distinctions are caused primarily by differences in the chemical composition of waxes. The waxes exhibit different mechanical properties: the wax of the lower layer is harder and stiffer than that of the upper layer. Moreover, crystals of the upper layer are very brittle and may be easily detached or broken to tiny pieces. The laboratory experiments showed that both wax layers reduce the attachment force of insects. Both layers lead to the reduction of the contact area of insects' feet with the plant surface. Additionally, crystals of the upper layer contaminate insects' adhesive organs.

P1722. The behaviour of *Drosera rotundifolia* L. (Droseraceae) trapping leaves in natural habitats

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Our aim was to investigate the influence of weather conditions and prey on the leaf-trapping behaviour of insectivorous plant *Drosera rotundifolia* (Droseraceae) in the natural habitat.

Continuous, non-manipulative observations on two sundew plants in Northern Russia and two plants in Middle Russia lasted 72 hours each. We estimated the shape of the leaf blade, the degree of slime secretion, percentage of curved margin tentacles and the number of captured prey for each of sixteen leaves chosen for the study.

Our observations show that different characteristics of leaf blade changed independently both on each other and on the presence of prey. The correlated behaviour of leaf blades from different plants also indirectly shows the absence of dependence between trapping leaves behaviour and prey. However, our experiments with artificial feeding in natural conditions show clear leaf reaction to the prey.

We propose that in natural undisturbed conditions changes of the leaf blade characteristics are casual and are augmented by the external factors such as relative air humidity, atmosphere pressure and presence of the prey on the leaf blade.

P1723. Respiration study in aquatic carnivorous plants: turions, traps and leaves

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Respiration at 4 and 20°C was compared in dormant autumnal turions of aquatic plants *Hydrocharis morsus-ranae* and *Caldesia parnassifolia* and aquatic carnivorous plants *Aldrovanda vesiculosa*, *Utricularia australis*, *U. ochroleuca* and *U. bremii* and after breaking imposed dormancy in the spring. Respiration rate at 20°C was 1.3-4.6 mmol/kg_{FW}.h in dormant turions and slightly increased (1.7-5.2 mmol/kg_{FW}.h) in the spring. However, non-dormant "turions" of (sub)tropical Australian *Aldrovanda* populations and American temperate *Utricularia inflata* and *U. purpurea* respired 7.6-11.3 mmol/kg_{FW}.h. Respiration rate of traps of 6 temperate aquatic *Utricularia* species (5.1-8.6 mmol/kg_{FW}.h) was 70-120 % greater than that in leaves or shoots. But net photosynthetic rate in photosynthetic leaves/shoots (40-117

mmol/kg_{FW}.h) exceeded that in traps by 7-10 times. Thus, *Utricularia* traps represent a great metabolic cost for the plants.

P1724. Carnivory Timed to Flowering in Triggerplants (*Stylidium*; Stylidiaceae)

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Australia's Triggerplants (*Stylidium* spp.; Stylidiaceae) 1) share habitat (wet, low nutrient soils) with accepted genera of carnivorous (*Drosera*, *Utricularia*, *Cephalotus*) and subcarnivorous/protocarnivorous (*Byblis*) plants and 2) possess glandular hairs which trap insects. Several lines of evidence were found to support the hypothesis that triggerplants are carnivorous: 1) trapping rates (prey m⁻²) for triggerplants and for accepted genera of carnivorous and subcarnivorous plants were very similar at three sites separated by 1500 km; 2) triggerplants, like *Drosera capensis* and unlike non-carnivorous plants, produced proteases in an inducible fashion when prey-trapping was simulated using yeast extract. This was true for plants flowering on soil as well as for plants flowering in vitro, the latter possibility excluding digestion by microorganisms on the plant surface. Therefore, triggerplants are carnivorous. It is very interesting that this carnivory is timed to just before and during flowering.

P1725. Photosystem-II damage and repair cycle in chloroplasts

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The mechanism of a photosystem-II (PSII) damage and repair cycle in chloroplasts will be discussed. Photo-oxidative damage to the PSII reaction center occurs in every organism of oxygenic photosynthesis. An elaborate repair mechanism has evolved that rectifies this irreversible photoinhibition and restores the PSII charge separation activity. The repair entails several enzymatic reactions for the selective removal and replacement of the inactivated D1/32 kD reaction center protein (the chloroplast-encoded psbA gene product) from the massive (>1,000 kD) water-oxidizing and oxygen-evolving PSII holocomplex. Evidence will be presented for the conformational status of PSII during D1 turnover. The composition of a PSII repair intermediate that occurs in the thylakoid membrane will also be discussed. This repair process is unique in the annals of biology; nothing analogous in complexity and specificity has been reported in other systems. Elucidation of the mechanism may reveal hitherto unknown reactions for the selective replacement of a protein from within multi-protein complexes. This may have applications in agriculture, medicine and other fields.

P1726. Impact of long-term salinity and oxidative stress on photosynthesis, growth, cellular antioxidants and medicinal quality of *Artemisia annua* L.

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In plants, salinity alters osmoregulation, ionic balance, stomatal behavior, photosynthetic rate and oxyradical generation. In response, plants try to cope with them by strengthening the cellular antioxidant system and status of osmolytes. We studied impact of the long-term salinity and oxidative stress on photosynthesis (P_n), growth, cellular antioxidants, proline and medicinal quality of *Artemisia annua* L. which yields artemisinin, effective against both drug-resistant and cerebral malaria-causing strains of *P. falciparum* and the cancer. Under salinity, *A. annua* showed reduced P_n and biomass accumulation. Contents of TBARS, proline, GSSG, DAs and activities of SOD, APx and GR increased but GSH and As declined. Artemisinin content increased initially (98%; 160 mM NaCl) but declined with progression of time. Thus, salinity causes oxidative stress in *A. annua*, reducing P_n , growth, Asc and GSH but increased contents of proline and glutathione (GSH+GSSG), and activities of antioxidant enzymes provide a certain level of tolerance. An increase in artemisinin content might be due to rapid conversion of its precursors by oxyradicals into artemisinin.

P1727. Photophysiological comparison of two green algae (*Nephroselmis olivacea*, *Pedinomonas minor*)

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We have examined two green algae with regard to different light conditions, like different growth illuminations, as well as short-time exposure to high light. The first one, *Pedinomonas minor*, is not able to de-epoxidize violaxanthin and therefore also shows a very small NPQ, mostly consistent of q_L . The second one, *Nephroselmis olivacea*, exhibits a typical violaxanthin xanthophyll cycle and also shows a strong NPQ, resulting from q_E .

Analyses of the carotenoid biosynthesis show that in both algae the pool of xanthophyll cycle pigments is raised following treatment with excessive light. The activation of the carotenoid biosynthesis in light is significantly higher in *Pedinomonas* than in *Nephroselmis*. The comparison of growth rates of both algae shows that *Pedinomonas* seems to be even better adapted to grow, under stronger illumination than *Nephroselmis*, although *Pedinomonas* is lacking the photoprotective mechanism xanthophyll cycle.

P1728. Functional Role of Pigments in Nigrescent Leaves

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Black-leaved plants should not exist. Theory predicts that the intense pigmentation would light-starve a leaf by absorbing energy that might otherwise be employed in photosynthesis. Although rare in nature, there are some species with extremely dark pigmented leaves. Not only do they survive but anecdotal evidence suggests that such plants are particularly robust against environmental stress. Mondo grass (*Ophiopogon planiscarpus*) was chosen because both green and black phenotypes were present in one species.

Experiments on optical properties as well as the functional significance of pigments, notably anthocyanin, were conducted on both morphs. Green and black mondo grasses were exposed to varying levels of increasing irradiance and temperature. Daily measurements of lipid peroxidation, antioxidant enzyme activity and total phenolic scavenging capacities were recorded.

The data suggests that the black morph provides a superior protection against photoinhibition and attributable to its total phenolic composition, possesses a higher scavenging capacity of reactive oxygen species.

P1729. Lipid influence on the enzymatic deepoxidation of xanthophylls

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We applied in vitro enzyme assays to analyse the influence membrane lipids on the deepoxidation reaction performed by the enzymes violaxanthin deepoxidase (VDE) and diadinoxanthin deepoxidase (DDE). Both enzymes exhibit high activities in presence of the non-bilayer forming lipids MGDG and PE. The DDE reaction rates saturate at lipid/pigment ratios (L/P) of 15, for optimal VDE-activity an L/P of 29 is required. These results correlate perfectly with the solubility properties of the two xanthophylls diadinoxanthin (Ddx) and violaxanthin (Vx) in these two lipids. Whereas Ddx is completely dissolved at a L/P of 15, for Vx a L/P of 29 is necessary. In the presence of bilayer forming lipids like DGDG and PC both enzymes revealed significantly decreased reaction rates, which cannot be explained by the strongly decreased substrate solubility in these lipids. Even at lipid concentrations that guarantee a completely dissolved substrate, reaction rates do not reach those observed in MGDG or PE. We therefore conclude that both the presence of non-bilayer forming lipids promotes substrate solubilisation and the structures provided are crucial for enzyme activity.

P1730. Terpenoid induction and oxidative damage in *Arabidopsis thaliana* plants

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Many plants release volatile monoterpenes (C10) and sesquiterpenes (C15) from their foliage, but the physiological role of these substances is not well studied. Different abiotic conditions

were used to test the effect of oxidative damage on the leaves (rosette stage) of *Arabidopsis thaliana* plants, and the changes in volatile profile were measured. The wild types WS and Columbia (Col-0) were exposed to high light, high temperature, and different concentrations of herbicides (like Paraquat, Bromoxynil, Acifluorfen, etc.) in order to see oxidative damage by measuring "in situ" superoxide and hydrogen peroxide accumulation (before visible symptoms appear), lipid peroxidation (MDA accumulation), and total H₂O₂ concentration. The use of *Arabidopsis* transgenic lines overexpressing various terpene biosynthetic genes and GUS-promoter fusion constructs allowed the manipulation of terpene levels to study their role during the plant stress response. This research will help in the understanding of the relation between terpenoids and the defence against oxidative stress.

P1731. How to localize subcellular glutathione in plants?

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Glutathione (GSH) is the most abundant member of the group of - glutamylcysteinyl tripeptides. It is considered to be a major redox buffer and to have a broad spectrum of functions in plants. While biochemical pathways involving GSH are known in some detail, there is considerable uncertainty about which tissues, cells and organelles are involved in glutathione metabolism.

In this study different methods (bimanes, immuno-fluorescence microscopy and TEM immunogold cytochemistry) were used to visualize and to investigate changes in the subcellular distribution of GSH in various plant tissues. In combination with digital image analysis quantification of GSH contents within different organelles was possible for the first time, showing steep gradients of glutathione levels between different cellular compartments (e.g. high levels in mitochondria vs. low levels in plastids). Differences in the distribution of GSH within tissues and cells during GSH modulation indicate that GSH concentrations are the product of an equilibrium between synthesis, degradation, usage, and short- and long-distance transport.

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P1732. Photoprotective mechanisms in foliose lichens have sufficient capacity to cope with a short-term high light stress

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Response of photosynthetic parameters of *Lasallia pustulata* and *Umbilicaria antarctica* to a short-term (30 min) high light (HL, 1 500 micromols m⁻² s⁻¹) stress was studied. Photoprotective mechanisms activated during HL exposure and consequent recovery were monitored fluorometrically (OJIP induction, quenching analysis, Chl fluorescence imaging), spectrophotometrically (APX - ascorbate peroxidase), and chromatographically (Z, V - zeaxanthin, violaxanthin, GSH - glutathione). HL induced an increase of Chl fluorescence levels O and J indicating decreased effectivity of electron flow in PS II. Fv/Fm and Φ_{II} dropped to 70 and 80 %, more apparently at thallus margins. In dark, Fv/Fm and Φ_{II} showed fast (1 h) and slow (10 h) phase of recovery. HL induced NPQ increment by the factors of 6 (*U. antarctica*) and 8 (*L. pustulata*). Fast decrease of NPQ during dark recovery related linearly to Z conversion to V. After HL treatment, total GSH decreased. Proportion of its oxidized form (GSSG) increased. In conclusion, hydrated *U. antarctica* and *L. pustulata* had sufficient capacity of photoprotective mechanisms to cope with HL stress. The latter species had a higher pool of total GSH.

P1733. Electron flows in chloroplasts under high temperature

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The aim of our investigation was the study of protective role of cyclic electron transport around PSII under high temperature. The effects of heat shock (40°C, 3 h) on the photosynthetic activity of 7-day-old barley seedlings were studied. Analysis of dark delay kinetics of chlorophyll a fluorescence (Fv) indicated thermoinduced suppression of linear electron flow due to increase of plastoquinone pool reduction. Taking into account the data on excessive reduction of plastoquinone pool and unchanged level of photochemical quenching of Fv it could be supposed that cyclic electron flow through PS II is accelerated by heat shock. This assumption was confirmed by the increase of content of Cy_{4HP} D₅₅₉

that mediates this electron transfer. Antimycin A as the inhibitor of electron flow from Cyt *b*₅₅₉ increased the plastoquinone pool reduction. The most strong effect in stressed leaves indicated that cyclic electron flow around PSII decreased level of plastoquinone pool reduction and protected electron transport chain from overreduction.

P1734. The induction of the synthesis of small heat-shock proteins of 14,5 and 16 kDa in suspension cultured tobacco cells in response to elevated ambient temperatures is mediated by changes in membrane fluidity and enhanced production of hydrogen peroxide

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The synthesis of heat-shock proteins (HSPs) is a fundamental reaction in all organisms to protect cells against heat injury and other adverse environmental impacts. With regard to the regulation of HSP synthesis under heat stress, the initial events that detect an increase in the ambient temperature and transduce it into a cellular signal are still a matter of debate. In this study, the involvement of physical properties of the cellular membranes and related oxidative processes in sensing a temperature upshift and inducing HSP synthesis were characterized in tobacco BY2 cells. We found that the overall increase in membrane fluidity by the addition of benzyl alcohol decreased the threshold temperature for the maximal activation of the synthesis of two small HSPs (14,5 and 16 kDa) in tobacco cells from 32°C to 28°C. This shift in HSP induction to lower temperatures by the membrane fluidizing effect of benzyl alcohol was paralleled with the enhanced production of H₂O₂. Our results provide evidence that alterations in the membrane fluid state take part in the perception of elevated temperatures and transmit the temperature signal by stimulating the generation of H₂O₂.

P1735. The NADP-ME family of *A. thaliana*: Specific biochemical properties and expression patterns suggest different roles for each isoform

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The *A. thaliana* genome encodes four NADP-malic enzyme isoforms. NADP-ME1-3 are localized to the cytosol while NADP-ME4 is localized to plastids as shown by assays of GFP fusion proteins. AtNADP-ME recombinant isozymes are functional and depicted differential kinetic and structural properties. NADP-ME2 exhibits the highest specific activity, while NADP-ME3 and 4 showed the highest activities towards NADP and malate, respectively. NADP-ME4 exists in equilibrium of active tetramers and dimers, while the cytosolic counterparts are present in higher oligomeric states. Comparative enzymatic activity assays in T-DNA insertion mutants and developmental studies of promoter activities indicate that NADP-ME2 is responsible for the major part of NADP-ME activity in *Arabidopsis*. NADP-ME2 and 4 show a constitutive pattern of expression, while expression of NADP-ME1 and 3 is under control of developmental and cell-specific signals. Studies with the insertional mutants indicates that NADP-ME2 is essential for survival under high light stress. Based on these findings, arguments for novel roles for some members of the NADP-ME family in *A. thaliana* are presented.

P1736. An in vivo anti-oxidant function of anthocyanins in red leaves is evident only when the pigments reside in the vicinity of the oxy-radical source

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Anthocyanins are considered as powerful anti-oxidants in vitro and such a function has also been proposed to explain their transient presence in some leaves. We argued that the likely anti-oxidant role should be enhanced when anthocyanins are located in the vicinity of oxy-radical formation, i.e. the leaf mesophyll cells and we designed experiments to this aim. We used plants with intraspecies variation in the express of the anthocyanic trait having anthocyanins either in the epidermal or mesophyll cells. We also

compared young red and mature green leaves. In all cases, oxy-radical production was photosynthetically induced by red light (which by-passes anthocyanins) after application of methylviologen. The protective function of anthocyanins was assessed by measuring photosystem II efficiency by chlorophyll fluorescence methods. The results indicate an anti-oxidant role only when anthocyanins reside within the mesophyll cells.

P1737. Winter wheat leaf glycolipid characteristics at the oxidation stress condition induced by hydrogen peroxide.

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The results of studies devoluted the winter wheat plant adaptive reaction to the the oxidation stress induced by hydrogen peroxide. The oxidation stress developing at exogenous hydrogen peroxide action was studied in wheat photosynthesizing tissues basing on lipid peroxidation product (primary - lipid hydroperoxides and secondary - TBA-active products) accumulation and antioxidative enzyme (superoxidedismutase and catalase) activity changing. It was found that oxidative stress intensity increased proportionally according to the oxidant concentration growth.

A set of hydrogen peroxide dose from low to high impact upon photosynthetic pigments and glycolipids was investigated. Hydrogen peroxide action peculiarities upon native systems and chloroplast isolated were studied.

Photosynthesizing tissue lipid-pigment complex and pro-antioxidation system parameters allow to screen winter wheat various variety for their tolerance against oxidative damage.

P1738. Free radicals in abiotically stressed plants investigated by EPR spectroscopy

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Free radicals are generated in normal metabolism and are controlled by redox reactions involving small antioxidant molecules and enzymes. Stress conditions affect the balance between free radicals and antioxidants, and stress-induced free radicals are usually inferred from changes in antioxidant levels in tissues. However, depending on the nature, magnitude and duration of the stress and plant genetic factors, these may be either increase or decrease. Direct investigation of free radicals in stressed tissues has been little investigated because of their transient nature and the lack of readily available analytical methods.

EPR spectroscopy is able to detect free radicals in biological tissues, and we report its use to study free radicals in tissues of plants that had been exposed to various abiotic stresses. Results show that stresses, in addition to influencing free radical levels *in vivo*, can affect the generation of stable free radicals during storage of processed products.

P1739. Effect of free air ozone fumigation on glutathione and total soluble sugars in adult beech trees.

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Total and oxidised glutathione (GSSG) and total soluble sugars were analysed in sun and shade leaves as well fine roots of beech trees (*Fagus sylvatica*) exposed to free air ozone fumigation (1xO₃, 2xO₃) in Kranzberg forest, Bavaria during the vegetation seasons of 2003 and 2004.

Changes in these parameters depended especially on seasonality and exposition of the leaves rather than ozone fumigation treatment (1xO₃ vs 2xO₃), which led to a significant difference only in the case of GSSG. The correlations between the particular ozone exposure (SUM0, AOT40, COU) and the parameters investigated are higher in shade than in sun leaves.

The fact that 2003 was a very dry year affected the parameters measured and their correlations with ozone. Apparently, drought and ozone in combination increased the oxidative stress for the trees. However, beech showed a resistance that was higher than expected.

P1740. Photochemical activity of two Mediterranean evergreen species under spring, summer and winter conditions.

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Plants of Mediterranean-type ecosystems are exposed during the year to adverse climatic conditions specially in summer and in winter. In this work the influence of seasonal climatic changes on thermal and photochemical processes was studied in *Quercus ilex* L. and *Laurus nobilis* L. Gas exchange and chl *a* fluorescence measurements were performed in situ on attached leaves at the actual seasonal temperature and relative humidity conditions and at 1000 PPFD. In both species A_N and g_{H_2O} were highest in May compared to February and July; the lowest values were found in July when leaf temperatures were the highest and precipitation was low. F_{PSII} decreased significantly in February compared to May and July both in *L. nobilis* and *Q. ilex*. F_{PSII} did not differ between May and July. The highest ETR/A_N found in summer for *Q. ilex* compared to *L. nobilis* indicated a major utilization of reductants to alternative sinks to photosynthesis. In February, the highest NPQ and the lowest ETR/A_N in both species suggest that thermal dissipation represents the main dissipative process of the excess excitation energy when photochemical pathways are reduced by low temperatures.

P1741. Chilling in the dark of two different pepper varieties (*Capsicum annuum*): a possible role of endophytes in stress response

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Two different pepper varieties (*Capsicum annuum*) a cold-tolerant var. Ziegenhorn bello (ZB) and a cold-sensitive var. Milder Spiral (MS) were cultivated at 23/18°C day/night temperature in a climate chamber.

Half of the plants were inoculated with endophytes (*Arthrobacter* sp. strain ZB04) isolated from from var. Ziegenhorn Bello.

One part of the plants was exposed to chilling temperatures during one night at 6°C and a second time one night at 4°C two weeks later.

Photosynthetic and chlorophyll fluorescence parameters were measured during chilling and the following days until full recovery. Chilling at 4°C had a much bigger influence on photosynthetic performance than at 6°C. Net rate of photosynthesis and actual photochemical capacity (F_v/F_m) were significantly reduced and ground fluorescence was increased shortly after chilling in both varieties independent of inoculation. Inoculation with the endophyte strain resulted in an increase in F_v/F_m in non-chilled plants. Analysis of carotenoid content of the leaves is under progress during submission of this abstract.

P1742. Analyzing biological diversity of plant ozone sensitivity can reveal mechanisms behind adaptation to polluted environment

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Here we address the natural variation of ozone (O_3) sensitivity/tolerance among *Arabidopsis* ecotypes. Commonly only single or couple of clones have been used to study O_3 response of different species. Since plant-specific responses may greatly differ, it is possible that different individuals of the same species utilize different biological processes to cope with oxidative stress generated by elevated O_3 . We have undertaken to screen the sensitivity/tolerance of about 100 *Arabidopsis* ecotypes collected throughout the world. This has revealed dramatic differences in O_3 -sensitivity among ecotypes adapted to different background environmental conditions. Selected most sensitive/tolerant ecotypes are used for further analysis aiming to identify which biological processes are behind the diversity in their adaptation and acclimation to climate change. These analyses include O_3 -induced changes in stomatal opening, concentration of antioxidants, induction of plant stress hormones and emission of volatile organic compounds. To map the genes that are activated by O_3 we use *Arabidopsis* DNA-arrays that enable simultaneous analysis of 25000 genes.

P1743. Function of a borate ester complex in the heterocysts of *Anabaena variabilis*.

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Boric acid (B), an essential nutrient of azototrophic cyanobacteria, is bound with high affinity to heterocysts (ca. 50 $\mu\text{mol/g}$ dry weight) at $\text{pH}>3$ in the presence of Ca^{2+} . The B-ligand was not extracted with methanol/chloroform and found to be resistant to alkaline treatment, but it was degraded by acid hydrolysis. Azototrophic growth was sustained by 0.2 μM soluble B. B complexed in senescent heterocysts could not be re-utilised. Due to mechanic instability of the contact between heterocysts and vegetative cells, azototrophic cultures were stressed by shearing (shaken vessels). A strict requirement of N assimilation on B occurred if cultures of the strain ATCC 29413 were agitated at low cell densities ($\text{OD} < 0.5$). This strain was able to grow on non-shaken B-free medium or in shaken cultures at a high cell density ($\text{OD} > 0.8$). The strain PCC 7119 was highly sensitive to shearing stress and did not grow azototrophically on B-free non-shaken medium. O_2 pressure did not effect B-requirement. It is assumed, that the B complex cross-links the heterocyst specific polysaccharide and is essential for metabolite transfer between the N- and C-assimilating cells.

P1744. Consequences of the expression of Glycolate oxidase in plastids of *A. thaliana* in the context of a biotechnological approach to reduce photorespiratory losses.

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During the course of the C2 oxidative pathway glycolate-2-P is recycled to glycerate-3-P and CO_2 and NH_3 are released inside the mitochondria. Due to the loss of fixed carbon photorespiration is considered as a wasteful process, although photoprotective properties were reported. We attempt to introduce catabolic pathways for glycolate in *A. thaliana* to release CO_2 inside the plastids. This would create autoregulatory cycles, which could attenuate photorespiration. In each cycle, the expression of three transgenes inside the chloroplasts is required. The first step of both pathways is catalyzed by glycolate oxidase (GO), yielding glyoxylate and H_2O_2 . GO plants are characterized by retarded growth and yellowish leaves. To elucidate whether H_2O_2 or glyoxylate is responsible for the observed phenotype, transgenic plants co-expressing GO and *E. coli* catalase (KatE) or GO and the second enzyme of each pathway were produced. Co-expression of KatE alleviates the GO phenotype and demonstrates that the chloroplastic endogenous machinery that detoxifies H_2O_2 is overloaded when KatE is not overexpressed. The analysis of these transformants will be presented.

P1745. Short Circuiting Photorespiration

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Transgenic tobacco plants have been generated with the aim of by-passing part of the photorespiratory cycle that converts glycine to serine. The plants have been transformed with the genes encoding glyoxylate carboligase (gcl: EC 4.1.1.47), which converts glyoxylate to tartronate semialdehyde and CO_2 , and hydroxypyruvate isomerase (hyi: EC 5.3.1.22), which converts tartronate semialdehyde to hydroxypyruvate. These enzymes are targeted to the peroxisome where they should 'short circuit' photorespiratory metabolism and decrease the energy requirements for photosynthesis by avoiding the release of ammonia. Under high CO_2 the plants had no obvious visible phenotype but under bright light and at ambient CO_2 , necrotic lesions developed on the leaf lamina of the transgenic lines. We report the molecular, metabolomic and physiological characterisation of these plants.

P1746. Single-cell C_4 photosynthesis in marine diatoms?

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Marine diatoms are estimated to fix as much carbon as all of the terrestrial rain forests. Their productivity is enhanced by a CO_2 -concentrating mechanism, but whether it is based on direct acquisition of inorganic carbon or on single-cell C_4 photosynthesis is still a matter of controversy. In a C_4 pathway phosphoenolpyruvate carboxylase (PEPC) could act as primary carboxylase in the cytoplasm and phosphoenolpyruvate carboxykinase (PEPCK) or malic enzyme (ME) as decarboxylase in the chloroplast, releasing CO_2 for Rubisco. Whole genome sequencing of the marine diatom *Thalassiosira pseudonana* has

revealed the presence of essential genes for a C4 pathway, including PEPC, PEPCK, NADP-ME and pyruvate-orthophosphate dikinase. These genes were cloned, and gene expression measured by quantitative RT-PCR. Furthermore, antibodies were raised against some of the corresponding proteins, and used for immunoblotting. To test the C4 pathway hypothesis, changes in transcript and protein levels were measured when the diatom was grown under different levels of C and N limitation.

P1747. The effects of neighbouring plants on stem height and diameter growth of *Chenopodium album*: wind or red/far-red?

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The effects of height of neighbouring plants on plant morphology were studied with individually potted *Chenopodium album* plants. A stand was established by arranging pots, where target plants were lifted or lowered to create height differences between targets and neighbours in the stand. Plants that were lifted to overtop neighbours reduced the rate of stem elongation and increased stem diameter growth. Lowered plants that were overtopped by neighbours increased the stem elongation rate without change in diameter growth. In the second experiment, we studied environmental factors that are responsible for growth modification. Two stands were established as in the first experiment, but in one stand black-painted artificial plants were used as neighbours to eliminate R/FR effects on targets keeping PAR similar to the other stand. In each stand, a half of targets were fixed to supporting sticks to reduce swaying by wind. Targets were lifted or lowered as in the first experiment. Growth modification was found only in the stand with natural neighbours even if plants were fixed to sticks. We conclude that R/FR rather than wind is responsible for the growth modification.

P1748. Costs and benefits of photosynthetic light acclimation in response to gap formation in a cool-temperate deciduous forest

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Shade leaves increase their photosynthetic capacity (P_{max}) in response to improved light environment after e.g. gap formation. To increase P_{max}, however, additional space is necessary for chloroplasts to increase their volume and leaves must be thick. The objective of the present study is to assess costs and benefits of photosynthetic light acclimation in natural conditions. We created gaps by falling canopy trees in a cool-temperate deciduous forest and studied the changes in photosynthetic characteristics of understory seedlings of *Kalopanax pictum* Nakai in response to gap formation. Data obtained in the field were applied to simulate photosynthetic rates under gap and understory conditions. We found that P_{max} increased by 27% after the gap formation, which led to 11% increase in daily carbon gain. This increase in carbon gain was greater than the amount of biomass invested for additional leaf thickness, suggesting that photosynthetic light acclimation is beneficial for growth and survival of understory seedlings when gap formation occurs.

P1749. Canopy distribution of physiological traits in 4 to 8-year-old hoop pine (*Araucaria cunninghamii*) trees in relation to season, fertiliser application and growth

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We examined the distribution of photosynthetic capacity (A_{max}), stomatal conductance (g_s), foliar N concentration, ¹³C and ¹⁵N, SLA, and maximum silhouette area ratio (R_{max}) in the canopy of 4 to 8-year-old trees two years after fertiliser application (0-300 kg N ha⁻¹) in summer and winter to establish the links between canopy gradients and the physiological traits.

Foliar N varied throughout the canopy and with fertiliser application and season. Patterns of foliar N, ¹³C and ¹⁵N were similar among fertiliser treatments and between seasons. Decreases in ¹³C and ¹⁵N along the branches were larger than those from upper to lower canopy. SLA in the upper outer was less than in the

lower outer canopy. ¹³C was positively correlated with SLA. R_{max} increased with canopy depth. A_{max} and g_s decreased with canopy depth and from the outer to inner branchlets with a positive correlation between A_{max} and g_s. Instantaneous WUE was negatively related to g_s. ¹⁵N significantly reflected N fertiliser uptake and distribution, and was significantly lower at wet than dry sites. The findings have implications for scaling up leaf-level physiological measurements.

P1750. Nitrogen allocation and the fate of absorbed light in 21-year-old radiata pine trees

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The fate of absorbed light may be influenced by N fertiliser given that the photosynthetic machinery accounts for more than half of the N in a leaf. We investigated the distribution of absorbed light to dissipation and photochemistry in N fertilised (N+) and not fertilised (N0) 21-year-old *Pinus radiata* D. Don trees in the lower, mid and upper canopy. Chlorophyll fluorescence, photosynthesis, Rubisco, chlorophyll, N, and needle morphology were measured. Dissipation (NPQ) did not differ between the upper canopies of both treatments, but was significantly lower in the lower and mid canopy of N+ trees than in the lower and mid canopy of N0 trees. The proportion of light energy used in photochemistry (PSII) did not differ between the treatments. N+ trees used photochemical energy more efficiently for CO₂ fixation (PSII/CO₂) than N0 trees. The amount of excess light was significantly higher in the lower and mid canopy of N+ trees than in the lower and mid canopy of N0 trees. We conclude that N fertiliser increases the susceptibility of *Pinus radiata* to photoinhibition in the lower and mid canopy.

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P1751. Fruit bearing branchlets are carbon autonomous in mature broad-leaved temperate forest trees

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The C source-sink relationship in fruit bearing branchlets of mature deciduous forest trees was manipulated, in order to evaluate the degree of C autonomy for fruit development. The treatments, which were applied by using a canopy crane, included girdling and half and complete defoliation of fruiting branchlets of *Carpinus betulus*, *Fagus sylvatica* and *Tilia platyphyllos*. Analyses of non-structural carbohydrate concentrations and a ¹³C tracer experiment were performed to identify situations of C imbalance and changes in C acquisition pathways induced by the treatments.

All three species exhibited complete C autonomy of fruiting at the level of whole, undisturbed branchlets, since neither the total infructescence biomass, nor the individual fruit mass decreased on girdled, un-defoliated branchlets. On girdled, 100 % defoliated branchlets, fruit biomass relative to controls was still about 50 % in *Carpinus* and *Tilia*, but only 20 % in *Fagus*, which reflects the small proportions of photosynthetically active infructescence tissues in the latter species. Interestingly, *Tilia* branchlets hardly imported C to compensate for assimilate loss after defoliation.

P1752. Direct Identification of Chloroplast Photosystems in *Aglaonema simplex* var. *metallica* by cryo optical fluorescence microscopy

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Cryofluorescence microscopy at minus 196 C with an Olympus long range objective at 0.7 N.A was used to observe isolated leaf chloroplasts of *Aglaonema simplex* var. *metallica*. This species and variety of *Aglaonema* is characterised by very dark green leaves.

Photosystem one is revealed by blue light excitation from a high pressure mercury vapour lamp to reside mainly in the intergranal regions with a peak at 730nm while Photosystem two is mainly in

the grana. with a peak at 680 nm..The 730 peak is absent at room temperatures

The chloroplasts have grana of up to 1.9 microns in width and are very long while the intergranal spaces are traversed with numerous membranes in a space approx.0.5 microns wide.This was revealed by bright field microscopy, followed by confocal fluorescence microscopy .

Standard Glutaraldehyde- formaldehyde fixation with post fixation in OsO4 showed the membranes and their disposition.

We suggest cryofluorescence microscopy is particularly useful in analysing the positioning of chloroplast photosynthetic pigment complexes.

P1753. Heat responses of pigment-protein-complexes in chloroplasts from callus and leaves of sugar beet

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For green callus and leaves of sugar beet we previously investigated the effects of chilling and/or strong light on the compositions of proteins (SDS-PAGE) and pigments (HPLC) in chloroplast thylakoid fractions [fragmentation of thylakoids by detergents and separation into at least 7 distinct bands by native green gel electrophoresis]. Here we report how this composition is affected when leaves (and isolated leaf chloroplasts) or green callus (and isolated callus chloroplasts) were kept at 40 - 50 °C for 30 - 120 min [standard conditions 120 min 50 °C] prior to thylakoid preparation. In most cases temperature induced alterations of protein composition are similar to effects of strong light and chilling stress and are most pronounced in pre-treated chloroplast suspensions. After heat stress of callus chloroplasts, the pigment concentration in presumed photosystem I is drastically reduced in favour of complexes with lower molecular weights. Appearance of zeaxanthin and reduction of the amount of lutein in almost all pigment-protein-complexes are the most striking effects of heat stress on leaves, callus and chloroplast suspensions.

P1754. Some aspects of desiccation tolerance in *Boea hygroskopica* and *Sporobolus stapfianus*

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The research presented was compiled through a series of collaborative studies between the University of Florence and the Universities of Pisa, Padova and Pavia (Italy) and Cape Town (South Africa). Desiccation-tolerant or 'resurrection' plants provide unique model systems to investigate metabolism in response to severe drought stress. Use of the desiccation-tolerant, dicotyledonous species *Boea hygroskopica* F. Muell (Gesneriaceae) and monocotyledonous species *Sporobolus stapfianus* Gandoger (Poaceae) as model systems was integral to the study of desiccation tolerance in the aforementioned collaborations.

Desiccation tolerance in 'resurrection plants' is dependent on the induction of various protective mechanisms during dehydration. Using the leaf material of both plant species, the present study focussed on aspects of desiccation tolerance pertaining to variations in drying conditions, to adaptive changes in cell structure and to antioxidant defence mechanisms. The accumulation of low molecular weight substances, with particular emphasis on aspects of sucrose and nitrogen metabolism, also formed part of the present investigation.

P1755. Change of photosynthetic capacity of *Alnus hirsuta* sapling grown in a free air CO₂ enrichment (FACE) system: comparing the proximate *Betula* spp. without symbiotic N₂ fixing micro-organism.

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To elucidate the differences of photosynthetic capacity between plants with N₂-fixing micro-organism (N₂-fixing plant) and non-N₂-fixing plants under high CO₂ concentration ([CO₂]), two-year-old *Alnus hirsuta* Turcz (N₂-fixing plant), *Betula platyphylla* var. *japonica* Hara and *B. maximowicziana* Regel (non-N₂-fixing plants) were grown with a free air CO₂ enrichment (FACE) system located

in northern Japan. [CO₂] was 500 μmol mol⁻¹ in FACE. Two soil conditions were prepared in each FACE with brown forest soil (high nutrient) and volcanic ash soil (low nutrient). All but "A. *hirsuta* in volcanic ash soil" and "B. *maximowicziana* in brown forest soil" showed the depression of photosynthetic capacity under high [CO₂]; i.e. down-regulation. As for A. *hirsuta*, the reason was mainly due to the excessive accumulation of starch in foliage. As for B. *platyphylla* and B. *maximowicziana*, the reason was mainly due to the reduction of Rubisco concentration. We concluded that both "A. *hirsuta*" and "B. *platyphylla* and B. *maximowicziana*" have a possibility to show down-regulation, but the reason is species specific characteristics as related to symbiotic microbes.

P1756. Identification of Metallothionein as a Heavy Metal Tolerance Mechanism in *Sphagnum magellanicum*

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Deposition of platinum group metals (PGMs), platinum, palladium and rhodium, from automobile catalytic converters has led to an increase in concentrations of these elements in the environment. Animals can tolerate some heavy metal accumulation by forming metallothionein (MT), metal-protein complexes. We studied *Sphagnum*'s mechanism of tolerance to PGMs and cadmium. *Sphagnum* was grown on Rudolph's media containing one of the following metals: Pt^{IV}, Rh^{III}, Pd^{II} (0.1, 1, 10, 100 ppm) or Cd^{II} (0, 0.1, 1.0, 5.0 ppm). After four weeks of exposure plants were harvested. Toxicological tests demonstrated significant DNA damage and decreases in length and biomass of plants in all metals and exposure concentrations. Metal accumulation in tissues was analyzed using a graphite furnace atomic absorption spectrophotometer. There was a positive correlation between metal concentration in media and in plant tissues. MT concentrations were determined by matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF). The induction of MT positively correlated to metal concentrations indicates that MT plays an important role in heavy metal tolerance.

P1757. Defense characteristics of deciduous broad-leaved tree seedlings grown under factorial combination of two levels of CO₂ and nitrogen

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Mixed broad-leaved forests are abundant in several kinds of herbivore species in northern Japan. Herbivores are depending on leaf quality. However, the leaf qualities are changing with increasing atmospheric CO₂ and nitrogen deposition. Four species of deciduous broad-leaved tree seedlings (alder, birch, oak & maple) were raised in a factorial combination of two levels of CO₂ and nutrients to examine the foliar defense traits. To evaluate defense capacity of four species, we used wild silkworms as a bioassay. Except for alder, survival and longevity were shortest in silkworms fed with leaves at high CO₂ and infertile condition while those were longest in leaves treated with ambient CO₂ and fertile. Longevity of silkworm fed with alder leaves was longer when alder seedlings at high CO₂ and infertile. Except for alder, results suggest that birch, oak and maple supported the CNB hypothesis. In alder, activity of symbiotic microbe would be enhanced by greater amount of photosynthates that they received from the host plants at high CO₂. In this, *Frankia* sp. would increase amount of nitrogen in plants, which improved food quality for silkworms.

P1758. Vegetation composition and ecophysiological traits in differently degraded stands of sandy grassland

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Under the continental climate of Carpathian basin semiarid grasslands represent one of the widespread vegetation. Due to overgrazing and other disturbances most of the semiarid grasslands in Hungary are the mosaics of more or less degraded

patches. Our work focused on the variations in the ecophysiological traits of the common dominant species in a moderately degraded (cover 60 %) and a strongly degraded stand (27%) of *Potentillo-Festucetum pseudovinae* community in summer. The reduction of soil moisture content species experienced significantly higher leaf water saturation deficit in the more open stand. Carotenoid pool was larger for all the species in the strongly degraded stand. The protective xanthophyll cycle pool was also higher in the latter stand and reached 30-33 % of the total carotenoid content. The potential photochemical efficiency (F_v/F_m) was lower for the monocot species. There was a decrease in the F_v/F_m values at noon, which was larger in the strongly degraded stand.

P1759. Above-ground and bellow-ground biomass in relation to grazing pressure on species-rich Dinaric karst pastures

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One of the most common activities on secondary grasslands is pastoralism which affects grasslands within grazing regime. Light, moderate and intensive grazing were applied on species-rich nutrient-poor calcareous pastures on Vremščica (Dinaric Karst, SE Slovenia) for 8 years. One location has been abandoned for 8 years. Two-seasons sampling on plots and sub-plots included plant composition and biomass. No significant differences were perceived in species richness by different treatments, while species composition and biomass were significantly affected by grazing regimes. On intensively grazed plots plant composition indicates habitat-generalists, which invest less bellow rather above-ground. On extensively grazed plots plant composition indicate on habitat-specialists, "karstic" sub-Mediterranean species, adopted to summer hydric stress, which invest much more bellow- that aboveground (rhizomes, bulbs). We could conclude that community assemblage with different plant functional types is affected by different grazing pressure.

P1760. Ecophysiological responses to simulated climate change (drought or nocturnal warming) in a semiarid forest-steppe in Hungary

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Ecophysiological responses to simulated climate change (drought or nocturnal warming) were studied in a semiarid temperate forest-steppe vegetation mosaic for three species representing three different functional types: *Cynodon dactylon* (L.) Pers. (perennial stoloniferous C4 grass), *Festuca vaginata* W. et K. (cold tolerant perennial C3 bunchgrass) and *Populus alba* L. (C3 deciduous tree, shrub-sized root sprouts colonize sand grasslands). Treatments caused only slight changes in plant physiological activity. There was a tendency for enhanced leaf photochemistry during the day in response to nighttime warming (~ 1°C increase of daily minimum temperature). Species reactions to extreme weather were more pronounced. The extremely dry and hot spring and early summer in 2003 caused lasting photoinhibition - and probably photodamage - for *F. vaginata* that led to an unprecedented dieback of the species in the area. No such changes were observed for the other two species. Different responses of the three functional types forecast compositional changes in the forest-steppe vegetation. Supported by EU FW5 EVK2-CT-2000-00094, NKFP-3B/0008/2002, OTKA T038028.

P1761. Leaf anatomical plasticity of *Brachypodium pinnatum* (L.) Beauv. in contrasting microhabitats in a semiarid forest-vegetation mosaic

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After felling natural xerothermic oakwoods in the forest-steppe loess regions of Hungary the perennial understorey grass *B. pinnatum* persists for decades from shade to full sun microhabitats. Variation in leaf anatomy was explored behind the species' highly plastic leaf photochemistry in response to habitat light regime in three typical microhabitats (full shade under oak canopy, half shade near shrubs, and in unshaded grassland) *in*

situ, and for plants transplanted between these microsites. Intervential lamina and mesophyll were 46-64% thicker in the grassland *in situ* than in oak subcanopy due to an additional layer and 25-32% greater height of mesophyll cells. Mesophyll thickness and the proportion of veins plus sclerenchyma were greatest in the grassland *in situ* exceeding that of full or half shade to full sun transfers. Outer bundle sheath tended to be the thickest in the grassland. Despite adjustments in leaf anatomy, plasticity at the shoot level and presumed cellular modifications may play the major role in medium-term light acclimation of *B. pinnatum* contributing to the species' success in secondary vegetation succession. Supported by OTKA T038028.

P1762. The role of rangelands of Uzbekistan in a global carbon cycling

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The vast areas of grazing lands in Uzbekistan are believed to have a large potential to sequester carbon and mitigate the greenhouse effect. Although arid and semi-arid ecosystems are known to have substantially lower productivity than forests, it was hypothesized that the vast landscapes of Uzbekistan dominated by rangeland ecosystem could be an important contributor to the "missing sink" (Nasyrov, 2000). Thus, the main objective of this study was to document the daily magnitudes and growing season dynamics of net ecosystem CO₂ exchange (NEE) in representative rangelands of Uzbekistan

The obtained results indicated that net growing season CO₂ fluxes were positive at rangeland monitoring sites in Uzbekistan.

The sagebrush-ephemeral semidesert site at Karnap, Uzbekistan sequestered 698 g CO₂ m² season.

Given the vast area of rangelands, this rate of carbon assimilation can turn Uzbekistan rangelands into a significant CO₂ storage sink, and they can greatly contribute to reducing the global warming.

P1763. The response of functional plant groups in a semi-natural grassland to long-term CO₂ enrichment

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The long-term responses of an oligotrophic semi-natural grassland to *in situ* free air carbon-dioxide enrichment (FACE) and different cutting frequencies were investigated since 1999. The area was neither artificially irrigated nor fertilised.

Significant stimulation of total above ground biomass production by elevated [CO₂] was only found after three years of CO₂ enrichment and again in 2003 when precipitation was only about 60 % of average precipitation.

Functional plant groups responded differently to CO₂ enrichment causing a clear shift in the botanical composition from 1999 to 2004 towards a higher proportion of legumes under elevated [CO₂].

In 2002 the lacking stimulation of above-ground biomass yield of the grasses could be explained by a down-regulation of V_{cm_{max}} in the dominant grass species *Bromus erectus*, whereas in 2004 carboxylation activity was not affected by elevated [CO₂].

Our results corroborate that in a nutrient-poor grassland community the legumes are the most competitive functional plant group. It has, however, to be taken into account that varying climatic conditions superimpose the response of the plant community to the treatments.

P1764. Ultrastructural alterations in thalli of lichen *Peltigera aphthosa* and in its isolated phycobiont *Coccomyxa* induced by continuous darkness

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The influence of the continuous lack of the light on the thalli of foliose lichen *Peltigera aphthosa* maintained in the air-dry condition was studied by the electron microscopy. The stress conditions induced in the lichen thalli the diminishing of contacts between phycobiont and mycobiont and the gradual degradation of their cells. These alterations indicated the evident decrease of the metabolic activity and were similar to those observed in the lichens under some other stress conditions or in the senescent lichen thalli. It supposed also some degree of delichenization of symbionts. The isolated cultures of *Coccomyxa*, phycobiont of *P. aphthosa*, in the dark conditions also underwent ultrastructural and

consequently functional changes in comparison with cultures maintained in the light. However, the alterations in these delichenized cells had some specificity and varied in the different nutritional media. The possible significance of the lichenization and of the water and nutritional supply for response of phycobiont to stress is discussed.

P1765. Growth inhibitory activity of metabolites from lichen mycobionts on photobiont

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Many novel metabolites, e.g. dibenzopyran, chromone, dibenzofuran, isocoumarin, were found in cultured mycobiont. Some mycobiont metabolites are different from lichen metabolites, and are thought to be often toxic to photobiont. Under mycobiont conditions, the normal metabolic pathway in the lichen seems to be inoperative, but the dormant metabolism is induced in the mycobiont, and is thought to have biological significance only for the mycobiont. In this paper, effect of metabolites isolated from mycobiont was examined on the algal growth in liquid medium with shaking for 14 days. The dry weight of *Trebouxia erici* cultured in BBM medium added 1 μmol of different metabolites were compared with background. Graphis lactone A, dibenzofurans, and chromones suppressed from 40% to 60% of the algal growth. On the other hand, graphenone, graphisquinones, and xanthenes did not suppress any algal growth. Biological significance of mycobiont metabolites was discussed.

P1766. Lichen photobionts from granite outcrops of the South Ukraine

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Investigations of 15 widespread lichens, growing on granite outcrops along Pivdennyi Buh River in the southern part of Ukraine using cultural methods are conducted. Species of *Trebouxia* genus found to be photobionts in 14 species of lichens. *Desmoccocus olivaceus* is photobiont of *Dermatocarpon miniatum*. It is established that in the South Ukraine such lichens as *Aspicilia hoffmannii*, *Candelariella vitellina*, *Parmelia somloensis*, *Ramalina polymorpha* and *Umbilicaria grisea* have one photobiont - *Trebouxia showmanii*, *Caloplaca aractina* and *Physcia tribacea* - *T. simplex*, *Physcia tenella* - *T. phycobiontica*, *Ramalina pollinaria* - *T. erici*. *T. showmanii* is the most widespread photobiont in plots studied. This species is characterized by the most stable growth in culture upon mineral medium. Besides, there were cases when *T. showmanii* formed mass growings on stones under natural conditions and in laboratory "wet camera". The morphology and the life cycles of algae isolated into culture were studied in details. All mentioned above isolated photobionts were for the first time found for the investigated lichens.

P1767. A lichen lectin binds to an alfa-1,4-polygalactoside urease located in the homologous algal cell wall.

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Recognition of an alga by a fungus is based on the production of fungal lectins with arginase activity. The algal receptor is a cell wall polygalactosylated urease. To study the binding of a lectin to its ligand, phycobionts from *E. prunastri* were used to digest the urease polygalactoside with α - and β -galactosidases. Arginase activity of the lectin was measured before and after incubation of algae with the protein. Sugars enzymatically removed from the cell wall were also measured and, finally, the binding of a FITC-lectin to isolated and enzymatically-treated algae was studied. Algae treated with α -galactosidase contain the lowest arginase activity and produce the highest amount of sugar after incubation with the enzyme. Phycobionts incubated with FITC-arginase retain fluorescence in their cell walls that can be removed by addition of galactose. Non-fluorescent walls were observed after incubation of β -galactosidase-treated algae with FITC-labelled lectin, recovering low values of fluorescence by disrupting algal cells. This implies that the lectin enter algal cells without the polygalactoside attached to the ligand.

P1768. Lateral CO₂ diffusion contributes to photosynthesis of homobaric leaves under alternating lightflecks

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The supply of leaves with CO₂ is generally considered from ambient air to the leaf interior but lateral gas fluxes inside leaves may also contribute to gas exchange. We speculated that, when only part of a homobaric leaf is illuminated, lateral flux of (respiratory) CO₂ from shaded to illuminated leaf areas may affect photosynthesis. Combined gas exchange and chlorophyll fluorescence imaging measurements were performed. Shaded leaves were alternately illuminated with a large and a small lightfleck. When stomatal conductance was low, calculated net assimilation rates (*A*) of homobaric leaves underneath the small lightfleck were substantially higher than underneath the large lightfleck, and electron requirement for CO₂ fixation (*e/A*) was clearly decreased by lateral CO₂ flux. All this was not observed in heterobaric leaves. Homobaric leaves can obviously refix respiratory CO₂ from (remote) shaded leaf areas and, consequently, enhance net CO₂ uptake under drought stress. We hypothesize that homobaric leaf anatomy was an advantageous trait in the past under conditions of low atmospheric CO₂ concentration, in particular, when combined with shortage of water.

P1769. Effect of elevated CO₂ on photosynthetic performance and dry matter accumulation in radish

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Radish plants (*Raphanus sativus* L.) were grown from seeds in climatic chambers under a light intensity of 750 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and two different CO₂ concentrations: 380 (ambient) and 680 (elevated) $\mu\text{mol}\cdot\text{CO}_2\cdot\text{mol}^{-1}$ air respectively. Photosynthetic rates (*A*₇₀₀) were significantly higher under elevated CO₂ over the whole vegetation period (up to +65%). In contrast to plants grown under ambient CO₂, a decrease in *A*₇₀₀ values in elevated CO₂ plants at the end of the cultivation period was obviously due to earlier senescence, which could also be seen from reduced carboxylation efficiency. As expected, water use efficiency (WUE) was significantly higher under elevated CO₂. This might mostly be due to lower transpiration rates in these plants (75-80% of ambient). After 45 days elevated levels of CO₂ resulted in an increase in total dry matter accumulation of +56% in harvested plants, in which assimilate translocation into the hypocotyl tuber was apparently higher (+70%) than into the leaves (+16%). Nitrate content of the tuber was lower under elevated CO₂ compared to ambient CO₂, whereas converse relationship occurred regarding ascorbic acid content.

P1770. Effects of Open Top Chamber CO₂ Enrichment (OTC) on leaf characteristics and photosynthetic capacity of grapevine (*Vitis vinifera* L.).

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Vines in a vineyard of the cultivar Touriga Franca were exposed to 2 concentrations of CO₂ (ambient and 500 ppm) using an open-top chambers carbon dioxide enrichment (OTC) system which allowed the exposure of the plants to constant CO₂ concentrations during summer of 2004. Some physiological and leaf anatomical characteristics were substantially affected by CO₂ enrichment. Net CO₂ assimilation rate was significantly increased, whereas stomatal conductance (*g*_s) was reduced in elevated CO₂, leading to improvements in intrinsic water use efficiency. The decrease in *g*_s was associated with a reduction of stomatal density and with direct effects of CO₂ on the stomata guard cells. Palisade and spongy parenchyma thickness were significantly higher in leaves grown under elevated CO₂ conditions. Those preliminary values may contribute to predict the magnitude of grapevine responses to climate change in the Mediterranean area.

P1771. Deuterium Isotopomer Distribution in tree ring cellulose: Separation between climate and physiological signals

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Plant-climate interactions have a strong influence on global carbon cycle. We study these interactions retrospectively using tree rings. The abundance of the stable hydrogen isotope deuterium (D) in plant material is influenced by climate and physiology. The D abundance of meteoric water carries a climate signal, which is partly passed on to plant material. Besides this, the D abundance of plant material depends on D fractionations of plant biochemistry, which constitute physiological signals. If these signals can be separated, climate and physiological signals can be obtained in parallel, allowing to infer plant-climate interactions. Deuterium Nuclear Magnetic Resonance allows to measure the D abundance of each C-H group of cellulose, which is the key to separating climate and physiological signals. Results from a laboratory experiment identify C-H groups in tree ring cellulose that carry climate and physiological signals. Results from a tree ring series show that climate signals (winter and summer precipitation) can be extracted. Experiments on longer tree ring series are in progress, to reconstruct climate and study long-term CO₂ fertilisation.

P1772. Competition dominates the responsiveness of juvenile European beech and Norway spruce to elevated CO₂ and/or O₃ concentrations

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A two-year phytotron study was conducted to investigate the intra- and interspecific competitive behavior of juvenile European beech and Norway spruce. Ambient and elevated carbon dioxide (CO₂) and ozone (O₃) regimes were applied as stressors for provoking changes in resource budgets, growth and allocation to facilitate the competition analysis. The hypothesis tested was that the responsiveness of plants to CO₂ and O₃ depends on the type of competition (i.e. intra- versus interspecific).

Spruce was a stronger competitor than beech as displayed by its higher aboveground biomass increments in mixed compared to monoculture. The responsiveness of the juvenile trees to the atmospheric treatments differed between species and was dominated by the type of competition: Spruce growth benefited from elevated CO₂ concentrations, while growth of beech suffered from the enhanced O₃ regime. In general, interspecific competition enhanced these atmospheric treatment effects, supporting our hypotheses. Our study stresses that competition is an important factor driving the development and responses of plants to elevated CO₂ and/or O₃.

P1773. Effects of elevated CO₂ on photosynthesis and biomass with radish.

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The relationship between the rate of photosynthesis/leaf area and biomass is still not clear. Elevated CO₂ should have positive effect on C₃ photosynthesis. We developed the system for simultaneous measurements of continuous monitoring of CO₂ exchange and growth analysis with whole plants to evaluate the impact of elevated CO₂ on whole plant photosynthesis and biomass (Plant Production Sci.7, 386-, 2004). A radish cultivar with a big storage root (White Cherrish) was grown under ambient (around 400 ppm) or elevated CO₂ (around 770 ppm). The effects of elevated CO₂ on the rate of photosynthesis with an individual leaf were also assessed. The results indicated that increase of dry matter by elevated CO₂ is due to higher photosynthesis/leaf area and bigger leaf area and higher rate of photosynthesis with increased leaf area does have positive effect on plant biomass of vegetative crops. Down regulation of photosynthesis with elevated CO₂ will be also discussed.

P1774. Effects Of Air Filtration On Egyptian Clover (Trifolium Alexandrinum L. Cv. Messkaw) Grown In Open-Top Chambers In A Rural Site In Egypt. I. Effects Of Ozone And Ozone Protectant Chemicals On Photosynthesis, Stomatal Conductance And Growth

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An Egyptian variety of clover plants (*Trifolium alexandrinum* L. cv. Messkaw) were either untreated (control) or treated with soil drench of ethylenediurea (EDU) at 50, 100, 150, or 200 ppm or

benomyl at 250, 350, 450 or 550 ppm for the whole growing season and sown in the soil either under Egyptian field conditions at a rural site in northern Egypt or in open top chambers receiving either charcoal filtered or no-filtered air. It was noticed that Both EDU and benomyl caused an increase in growth parameters and provided some protection against O₃ visible injury symptoms, with EDU having better protection than benomyl. Moreover, photosynthetic rates were increased in treated plants while stomatal conductance did not show any significant difference from control plants under OTCs conditions. It is clear from the results of the present investigation that benomyl and EDU would be useful tools to assess the effects of ambient O₃ on plants under Egyptian field conditions. The implications to Egyptian agriculture were discussed.

P1775. Influence of elevated atmospheric CO₂ concentration and temperature (+3°C) on photosynthesis, water relations, ontogenetic development and yield of *Triticum durum* L. and *Vicia faba* L.

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Plants of *Triticum durum* L. and *Vicia faba* L. were grown at present climatic conditions (365 mg CO₂.m⁻³; CS 2002) and at predicted future climatic conditions (700 mg CO₂.m⁻³; +3°C; CS 2100) in two growth chambers in which seasons were simulated. Additionally, plants were subjected to different water regimes (45% and 75% of WHC). Growth in CS 2100 significantly increased light saturated photosynthesis of both species. Leaf conductance of *T. durum* plants grown in CS 2100 was reduced in spring and increased in early summer and summer. Contrastingly, *V. faba* showed a decrease of leaf conductance throughout ontogeny. Plant development and biomass accumulation were accelerated in both plant species grown in CS 2100. In *T. durum* the ontogenetic difference between the climatic scenarios increased through ontogeny and finally reached 18 d at dead ripeness, causing a shortening of the various ontogenetic stages, especially the grain filling duration, resulting in a lower grain weight. In *V. faba* a difference of 7 days was already observed at vegetative growth stage and remained constant. An increase of seed dry weight per plant in CS 2100 was only found under mild drought stress.

P1776. Nitrogen use efficiency of a poplar plantation is increased under elevated CO₂

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Growth of trees is largely regulated by N availability. When plants are exposed to elevated CO₂ concentrations they often exhibit enhanced growth with increased biomass accumulation, which in the long-term can result in an increased N uptake from the soil. In fact, N and C cycles are usually strictly related within plants and N metabolism is regulated by signals that are derived from C metabolism (Stitt et al., 1999). In this study we will present data from the POPFACE experiment where a short rotation poplar plantation has been exposed to a CO₂ concentration of 550 ppm from planting to canopy closure. In particular we found that elevated [CO₂] treatment induced a decrease of N concentration in all plant tissues and as a result of the the increased biomass production, the N use efficiency, that is the biomass produced per unit of N in the plant, was increased under elevated [CO₂]. Despite total N content in the plant tissues was not different between elevated and ambient [CO₂], N depletion in the soil was evident under elevated [CO₂] suggesting some possible mechanisms of N immobilization.

Stitt M, Krapp A (1999) *Plant Cell Environ* 22:583-621.

P1777. Sink-limitation as a possible reason for limited growth response of sugar beet under free air CO₂-enrichment ?

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Effects of elevated CO₂ concentrations (550 ppm) on a crop rotation are investigated within the free air carbon dioxide enrichment (FACE) experiment at Braunschweig, Germany (1). During two growing seasons it has been found that growth and yield responses of sugar beet plants to the high CO₂-treatments were small in comparison to what could be expected in comparison to responses of other crop species. In addition, at the end of the growing season, a decrease of leaf area index (LAI) was observed under FACE conditions. Partial shading of the sugar beet canopy prevented the decrease of LAI under FACE and resulted in a higher growth stimulation than under full sunlight conditions. The results provide evidence for a possible sink limitation of sugar beet growth under future atmospheric CO₂ levels.

(1) Weigel, HJ, Dämmgen U (2000): The Braunschweig Carbon Project: Atmospheric Flux Monitoring and Free Air Carbon Dioxide Enrichment (FACE). *J Appl Bot* 74: 55-60.

P1778. The role of polyamines during induction of photosynthesis

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Before re-illuminated leaves, protoplasts or chloroplasts achieve a steady state photosynthesis there is an induction period lasting from a few seconds to a few minutes. We have followed induction both polarographically and physicochemically in the unicellular eucaryote *Scenedesmus obliquus* and its mutant that lacks light harvesting complex (wt-LHC) and we demonstrate that artificially altered endocellular polyamine levels affects induction. Specific fluxes and activities were also evaluated non invasively with JIP-Test. High endocellular levels of the polyamines delay photosynthetic induction and reduces maximum photosynthesis, estimated as rate of oxygen evolution. In the case of wt-LHC mutant polyamine-treated cells showed no inhibition during photosynthetic induction. The role of polyamines on the light harvesting complex size during induction is discussed.

P1779. The photochemical use of absorbed light by plants

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To gain information on the current photochemical use (PCU) in leaves, signals of chlorophyll fluorescence were taken to interpret the responses to environmental stress. Predawn Fv/Fm values were used to quantify available photochemical capacity at the beginning of the day (100%). Measurements were taken at different time intervals to determine the capacity of recovery of electron transport on upper and lower leaf surface.

Green house grown radish plants showed surprisingly high PCU (up to 35%, calculated from values of light exposed and 15' dark adapted leaves) under "photochilling" conditions (high light, low temperature). We found, however, also a great portion of photoinhibited centers (17% on upper surface), which did not manage to fully recover within 20 hours. Heat stress under low light conditions resulted in a severe inactivation of photosystems (more pronounced on lower surface) without considerable recovery.

Well watered rice plants exhibited 7 fold higher PCU than water stressed plants. This also corresponded to higher electron transport rates (ETR) in the former, nevertheless combined with a slightly higher amount of photoinhibited centers.

P1780. Determination of rhizome dry matter content of *Iris pumila* L. using a non-destructive method

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Commonly used method for determination of dry matter content (which includes measuring of fresh, water saturated and dry mass of plant organs) could be unrecommendable in some cases. Classic approach with harvesting of rhizomes (oven-drying) could have undesirable consequences on the abundance and number of species on its natural site. In our investigation we observed the

biomass production of rare and endangered *Iris pumila* L. in Deliblato Sand (Serbia). The fresh rhizomes of *I. pumila* were divided into smaller parts, and planted on the same site. The initial concentration of dry matter in this parts of rhizomes, was indirectly determined using a volumetric method (measuring the fresh mass and volume of rhizomes). On the basis of our results from measuring rhizomes of different clones and different age, we concluded that fresh matter concentration is a good predictor of dry matter content of this plant organ. Values of fresh matter concentration (g/cm³) obtained from volumetric method were highly correlated ($r=0.91$; $p<0.05$) with values of dry matter content (g/g) obtained with classic approach of measuring in control group of rhizomes.

P1781. Dynamics of two Mediterranean coastal ecosystems using NDVI from satellite images

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Among the modern methods for monitoring ecosystem dynamics the use of satellite imaging has been attracted special interest during the recent years. The Normalized Difference Vegetation Index (NDVI), which can be extracted from such images, has been proved to show good correlation with plant productivity. NDVI represents a measure of leaf chlorophyll content and may be correlated with photosynthetic activity, Leaf Area Index (LAI) and the Fraction of Absorbed Photosynthetically Active Radiation (FAPAR). Consequently, NDVI may be used for monitoring ecosystem dynamics, as it may fluctuates due to intra-annual growing processes (e.g. leaf burst or senescence of deciduous plants), or due to inter-annual climate variability, either natural or anthropogenic. In this study, NDVI was used for monitoring the dynamics of two adjacent but different coastal ecosystems: a phryganic one, dominated by the semi-deciduous shrub *Phlomis fruticosa* and a forest with *Pinus pinea*. NDVI has been correlated with precipitation - the main stress factor in Mediterranean type ecosystems - in a 7-year time series.

P1782. Photosynthetic pigment contents in twigs of 24 trees assessed by *in vivo* reflectance spectroscopy consistently indicate low chlorophyll levels but high carotenoid/chlorophyll ratios

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Preliminary trials indicated that spectral reflectance indices used to assess non-destructively photosynthetic pigment levels in leaves can be safely used for twigs as well, provided that twig periderm could be easily peeled off. We hypothesized that pigment levels in twigs should be compatible to the shade adaptation syndrome, due to the highly light absorptive periderm. Therefore, we compared selected indices obtained from leaves and twigs of 24 species. Twigs consistently displayed lower chlorophyll but higher carotenoids/chlorophyll ratios. When the exposed and shaded sides of twigs were compared, no differences were found. The results were unexpected, indicating that shade may not be the primary factor shaping cortical pigment profiles. Based on the sparse available literature, we suggest that red light enrichment and/or the extremely high CO₂ levels in the stem interior may pose additional photoselective and/or photoprotective needs, necessitating increased carotenoids/chlorophyll ratios.

P1783. Dynamics of leaf and root growth measured by digital image sequence processing - endogenous control versus environmental impact

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For their outstanding importance, analyses of internal growth regulations and environmental impact thereon are long-standing topics in plant sciences. However, only with recent technological advancements, it has become feasible to study the dynamics of plant growth in temporal and spatial scales that enable to establish a framework of multi-scale models, linking macroscopic growth with molecular events. Digital image sequence processing has been used to calculate maps of growth rate distribution from time-

lapse videos of expanding roots and leaves of dicot plants with high spatio-temporal resolution. The techniques have successfully been applied to a wide variety of species and questions, ranging from investigating the effects of external nutrient availability on growth dynamics in maize roots to identifying the genes controlling diel growth patterns in *Populus* leaves. On the basis of these results, the hypothesis is developed that the relative importance of endogenous control versus environmental impact on growth dynamics depends on the spatial and temporal variation of the environmental conditions to which the tissue is exposed.

P1784. Function of plant body odour: non-invasive assessment through phytohormones

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Plant hormones mediate phenotypic plasticity in plants. Upon attack by herbivores plants produce phytohormones that are involved in signal transduction resulting in the emission of complex odour blends that may consist of hundreds of compounds. One of the effects of these volatiles is the attraction of natural enemies of the herbivores as a kind of 'bodyguards'. Which of the volatiles released by plants function as such is mostly unknown, especially because it is difficult to make synthetic forms of the plant's bouquets due to the large number of compounds and the unavailability of many of their constituents.

Addressing the ecological role of individual plant volatiles has been greatly enhanced by elucidating the involvement of phytohormones in the induction. This provides an important tool for understanding the contribution of individual plant volatiles in interactions with arthropods. In addition to an important contribution to fundamental knowledge, also novel developments in environmentally benign pest control may be gained. Phytohormones may be used to prime plants so as to enhance their induced defences in response to herbivory.

P1785. Spectral Reflectance Compared with Fluorescence and Absorption Measurements of Seagrasses

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Comparison of three non-intrusive, efficient measurement techniques for physiological responses were carried out sequentially on the same seagrass specimens. The *Thalassia testudinum* specimens (12 specimens each measured 3 times on each of 2 blades) were incubated 24 hr at 32 and 16 ppt. The same specimens in sequence were measured first for fluorescence (after dark-adaptation), then absorbance, finally spectral reflectance. Results showed that the spectral reflectance low salinity measurements were statistically-significantly different from normal seawater; however, absorbance and fluorescence measurements were not significantly-significant (high standard error). Additionally, spectral reflectance indices (first derivative, NDI, SIPI) delineated effects of the stressor. The questions of recording the entire spectrum of values via spectral reflectance rather than two or three spectral lines of values in fluorescence and absorbance measurements will be discussed, as will differences expected from various optical sensor diameters.

P1786. Leaf cooling curves: a method for estimating leaf temperature in the sun and its application.

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The evolutionary significance of leaf size is commonly attributed to temperature regulation, with theory predicting that small leaves are adapted to hot, dry climates. This is because small leaves have small boundary layers and thus rapid heat transfer, so should therefore be more thermally coupled to ambient temperature than large leaves. A similar theory can be applied to lobed *versus* entire leaves of the same area. Understanding how different leaves cope with heat is important as high leaf temperatures can lead to leaf or plant death. Thermal imagery is increasingly used to estimate foliage temperature, yet a common problem is that leaves cannot accurately be measured in full sunlight because reflectance from the leaf can distort its true temperature. We have developed a method for estimating leaf temperature in the sun using thermal imagery to generate cooling curves. From these curves, the time constant for cooling, τ , can be calculated, from which the original

temperature of the sunlit leaf can be extrapolated. Consistent with leaf boundary layer theory, we have found that τ varies with leaf morphology.

P1787. Thermography- A Potential Technique in Plant Physiology Research

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Infrared thermography has been applied to measure leaf/canopy temperatures giving information of the energy balance of plant leaves that correlates very strongly to the rate of evaporation which in turn is dependent on stomatal conductance. The Iwanow effect (a rapid initial opening of the stomata before a longer-term closure in conditions of water supply insufficiency) was assessed on fully-developed wheat leaves which were cut off from their stems. Infrared thermography was also performed on a field experiment for comparative assessment of presymptomatic visualization of phytotoxicity. A higher average temperature was obtained in one of the fungicide treatments in comparison with the control and a second fungicide treatment for all wheat cultivars screened. Infrared thermography has been proved to have the adequate sensitivity for studies on stomatal dynamics on single leaves. It could be also adapted for use in the field as it seems to be precise enough in outdoor conditions. As a remote technique it does not interfere with stomatal conductance when measurements are being carried out and allows the semiautomatic screening of large areas in a very short time.

P1788. Possible amplification of mature mRNAs in single cells of tomato callus aggregates by direct RT-PCR of cytosolic contents suctioned out with a micropipette

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Two-step PCR (RT-PCR and nested PCR) was applied to single-cell RT-PCR for detecting gene expression *in situ* in single selected cells of leaf-derived callus aggregates of tomato (*Lycopersicon esculentum* Mill, cv. Moneymaker). The cytoplasm from one cell was removed with a micropipette using a light microscope and directly subjected to RT-PCR, followed by nested PCR. This method to remove cytosolic contents prevented the introduction of genomic DNA into the RT-PCR, and only intron-spliced products were amplified when the intron-containing genes were used as PCR targets. In addition, transcription of the intron-free gene was possibly detected by simultaneously tracing the intron-containing and intron-free genes using mixed primers for the targeted genes. The present study indicated that some stimuli-activated genes, such as the acidic extracellular chitinase and TLC1-retrotransposon long terminal repeat genes, were constitutively transcribed in tomato callus cells.

P1789. Long and short-term responses to contrasting irradiances in *Copaifera langsdorffii* Desf. (Caesalpinaceae)

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It was compared long-term responses of growth and leaf nutrient content in potted young plants of *Copaifera langsdorffii* at open and shaded areas without water stress. When young plants were transplanted to field under the same irradiance conditions the photosynthesis (A), leaf water potential (Y), and potential photochemical efficiency (Fv/Fm) were measured during daily courses in wet and dry seasons to point out short-term responses of carbon and water balances. The greater biomass accumulation, height, and total leaf area were linked with higher photosynthetic capacity on area basis in open area. All young plants survived in shade and it seems to be related with fast and high biomass accumulation in root. Net photosynthesis value in shade increased periodically when bunches of direct light (sunflecks) reached the leaves. The sunflecks took place in larger amount at dry period, but they were more effective for carbon assimilation in the rainy season. The ability of young plants to persist by long-term in shade could explain the wide distribution of *C. langsdorffii* in different Cerrado physiognomies.

P1790. Photosynthetic Pathways in Bromeliaceae, Based on Carbon Isotope Ratios of 1873 Species

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In the largest single-family survey to date, photosynthetic pathway was investigated in 1873 species of Bromeliaceae, representing ca. 65 % of all species and 55 of the 56 genera, using plant carbon-isotope ratio (d13C). Whereas 902 species showed d13C values typical of C3 plants (values more negative than -23 ‰), 827 showed d13C values typical of plants performing crassulacean acid metabolism, or CAM (values less negative than -20 ‰). A small number (144) of species showed intermediate d13C values (-20 ‰ to -23 ‰). The distribution of d13C values was strongly bimodal, with clusters around -26 ‰ and -13 ‰. These results suggest that about 44 % of all bromeliad species may utilize the CAM pathway as their principal mode of photosynthesis, including members of nine genera not previously known to contain CAM species. Additional species with intermediate and C3-type d13C values may also possess the capacity for some nocturnal CO₂ uptake via CAM. There was a positive correlation between altitude and d13C for species showing C3-type d13C values only, the first demonstration of a differential response of C3 and CAM plants to altitude with respect to carbon-isotope composition.

P1791. Life-time measurements of net CO₂ exchange in the C-3-CAM species *Mesembryanthemum crystallinum*

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Net CO₂ exchange of the annual, halophytic species *Mesembryanthemum crystallinum* was measured continually throughout the entire, approximately 5-month life cycle of plants grown either in the presence of 400 mM NaCl in the hydroponic root medium from day 34 onwards, or maintained in a non-saline root medium. In plants exposed to 400 mM NaCl, about 30 % of life-time carbon gain of whole shoots was derived from dark CO₂ fixation involving crassulacean acid metabolism (CAM). By contrast, in non-salt-treated plants life-time carbon gain of shoots resulted almost exclusively from CO₂ fixation in the light and nocturnal carbon balance remained negative on all measuring days. These results are not consistent with the widely expressed notion that the induction of CAM by high salinity in *M. crystallinum* merely represents an acceleration of normal plant-age related developmental processes. Rather, utilization of the CAM pathway for carbon acquisition in *M. crystallinum* is overwhelmingly, if not fully, under environmental control.

P1792. Characterization of *Arabidopsis thaliana* transgenic lines expressing high levels of maize NADP-malic enzyme.

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Arabidopsis thaliana (Col-0) was transformed with the maize chloroplastic NADP-malic enzyme (NADP-ME) cDNA under the control of 35ScaMV promoter. This enzyme catalyzes the decarboxylation of malate, generating pyruvate, CO₂ and NADPH. The homozygous line with the highest expression level showed a 30-fold increase in NADP-ME activity. Plants grown under normal light intensity showed no major morphological differences compared to the wild type line. However, chlorophyll content as well as photosynthetic performance indicators like CO₂ assimilation, Fv/Fm and PSII were significantly lower for transgenic lines. These results suggest that the presence of a highly active NADP-ME in chloroplasts of *A. thaliana*, could have important effects on the reductive power balance of this organelle, diminishing the efficiency of the overall photosynthetic process. The transgenic plants obtained in the present work represent a useful tool to understand the regulation of plastid redox metabolism.

P1793. Photosynthetic activity and growth of seedlings of four oak (*Quercus*) species grown in a combination of light and water regimes

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Photosynthesis and respiration rates determine the carbon gain and plant growth. In this paper we present the results of an experiment growing seedlings of four Mediterranean *Quercus*

species (*Q. canariensis*, *Q. ilex*, *Q. pyrenaica* and *Q. suber*) under contrasted conditions of light and water in a glasshouse. Maximum photosynthetic activity (A_{max}) was higher for *Q. pyrenaica* (mean of 8.45 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and lower for *Q. canariensis* (5.57 $\mu\text{mol m}^{-2} \text{s}^{-1}$). There was a significant decrease of A_{max} for all the species when grown under deep shade (mean of 5.02 $\mu\text{mol m}^{-2} \text{s}^{-1}$), compared to full light treatment (7.92 $\mu\text{mol m}^{-2} \text{s}^{-1}$). There was also a significant decrease of A_{max} for all species when grown under drought stress (mean of 4.06 $\mu\text{mol m}^{-2} \text{s}^{-1}$), compared to watered plants (9.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Interestingly the interaction of both factors, light and water, on the A_{max} was also significant. The photosynthetic values are related with other physiological (stomatal conductance, respiration, WUE) and morphological (SLA) variables, and their role determining relative growth rate of seedlings is investigated.

P1794. Additional synthesis of starch from sucrose in leaves of *Arabidopsis* in the light

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Accumulating during daytime starch is converted in the night into sucrose and consumed in respiratory, biosynthetic and transport processes. However in the light the degradation and conversion of starch are blocked. In pulse chase experiments with wild type plants and starchless mutants *pgm* or *adg1* of *Arabidopsis* an increase of starch radioactivity during chase in nonradioactive medium in the light was detected. These findings suggest that starch was additionally synthesized from labeled cytosolic soluble photosynthates, preferentially from sucrose. Radiogasometric studies of gas exchange have revealed that sucrose is consumed also in photorespiratory decarboxylations. To be involved in photorespiration the products of sucrose degradation must be transported from cytosol into chloroplast. We presume that derived from sucrose hexoses are transported into chloroplast by hexose transporter and phosphorylated there in hexokinase reaction. The phosphorylated hexoses may be consumed either for additional synthesis of starch or incorporated into the reductive pentose phosphate cycle and, via this cycle, into the glycolate cycle.

P1795. Patchy acclimation of photosynthesis due to corresponding spatial heterogeneity in epidermal anthocyanin accumulation.

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In the present study, we have examined the hypothesis that an epidermal anthocyanin screen predisposes the underlying chloroplasts to changes related to a shade acclimation syndrome. Variegated leaves of the tropical understory species *Coleus blumei*, which have regions with high chlorophyll density and are covered by anthocyanin-rich or anthocyanin-less epidermis, were used. The anthocyanin patches were consistently correlated with lower chlorophyll a/b and lower carotenoid/total chlorophyll ratios. In addition, imaging of chlorophyll fluorescence parameters (using a red LED source for both measuring, saturating and actinic illumination, i.e. measuring photosynthesis by light which bypasses the anthocyanin screen) indicated slightly, but statistically significant, lower maximum electron transport rates (ETRs) along photosystem II in the red patches. The results confirm the shade adaptation hypothesis of red leaves.

P1796. Control of respiration in photosynthesizing leaves of C-3 plants by irradiance and CO₂

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In leaves of seven C-3 species respiratory CO₂ fluxes from primary (R_p) and stored (R_s) photosynthates and total rate of respiration (R_t=R_p+R_s) were measured in the light under steady state photosynthesis. Respiration in the dark R_d was determined 40 min after switching off the light. Values of R_r were the same at low and high light, 130 and 650 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Under high light R_p was 25% higher and R_s by the same extent lower than under low light. R_d also increased after exposure to high light. R_r was lower than R_d both at low and high irradiances. Light curves of CO₂ efflux indicated that inhibition of R_s was initiated at very low irradiances, the initial rapid decline in the region from 0 to 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was followed by a slower decrease at higher irradiances. In leaves of *Helianthus annuus* the inhibition of R_s was at 3000 $\mu\text{L L}^{-1} \text{CO}_2$ less pronounced than at 360 $\mu\text{L L}^{-1}$. In leaves of *Secale cereale* the increase of CO₂ concentration from

300 to 2300 $\mu\text{L L}^{-1}$ did not change Rr but resulted in 2-fold decrease of the contribution of Rrp with concomitant increase of Rrs. Under very high CO_2 , 30 mL L^{-1} , the inhibition of Rrs by light was removed.

P1797. Effect of thinning on photosynthetic acclimation and its relation to leaf nitrogen in young stands of *Chamaecyparis obtusa*

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In order to distinguish carbon fixation benefited from forest management from non-human factors, we studied the changes in photosynthesis after thinning and analyzed its relation to leaf nitrogen. The study site was a 10-year-old *Chamaecyparis obtusa* stand (36°3'N, 140°7'E) with 4.24 m high. The forest density was 2778 trees ha^{-1} . Half of the trees was cut one by another in the thinned treatment in May 2004. The photosynthetic responses to CO_2 concentration were measured *in situ* in leaves in July and October to estimate V_{cmax} . Thinning improved light condition in the lower and middle crowns but not in the upper crown. Nitrogen content per area (N) did not change after thinning at all crown positions. Thinning caused an increase in V_{cmax} only in lower crown in June, and in both middle and lower crowns in October. The slope of the linear regression between N and V_{cmax} was different between thinned and non-thinned treatments in October, but not in June. These results indicate that photosynthetic acclimation in the first year after thinning might mainly depend on nitrogen partitioning within photosynthetic machinery, but not on nitrogen reallocation between leaves.

P1798. Effects of salt stress on soluble proteins, proline and carbohydrates content of two tomato cultivars under *in vitro* culture condition

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The effect of NaCl stress on soluble proteins, proline and carbohydrates of two tomato (*Lycopersicon esculentum* Mill.) cultivars Isfahani and Shirazy were studied. Seeds were germinated on medium containing only water agar and then transferred to MS medium supplemented with different concentrations of NaCl (0, 40, 80, 120 and 160 mM) for 24 days. Increasing of salinity resulted in increasing of soluble proteins in stem-leaf of cv. Isfahani but decreasing in cv. Shirazy. Soluble proteins in roots of both cultivars showed some variations. When the concentration of NaCl in the medium was increased, proline contents of stem-leaf and roots of both cultivars was increased significantly. However, cv. Shirazy showed higher amount of proline level. Total carbohydrate in stem-leaf and roots in both cv. Shirazy and cv. Isfahani was increased in response to increasing of salt concentration. It can be concluded that, proline content in compare with the soluble protein and carbohydrate level is genotype dependent and can be a reliable criteria for evaluation of salt tolerance in tomato cultivars Isfahani and Shirazy.

P1799. Polyamines regulate the sensitivity of photosynthetic apparatus to UV-B radiation through changes in the molecular organization of LHCII

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The sensitivity of the photosynthetic apparatus to UV-B is influenced by the size of LHCII, which is regulated by thylakoid-associated polyamines, especially putrescine (Put) and spermine (Spm). In *wt* cultures of *Scenedesmus obliquus*, a decrease in the Put/Spm ratio in thylakoids led to the increase of LHCII size and, consequently, to a higher sensitivity of the photosynthetic apparatus to UV-B. Inversely, an increase in the Put/Spm ratio conferred a higher degree of tolerance to UV-B radiation. Experiments with the mutant *wt-LHC* (without LHCII) showed that the changes induced by UV-B in the photosynthetic apparatus were less pronounced as compared to the *wt* cultures, but the ability to recover the photosynthetic ability was significantly decreased. The results highlight the LHCII importance in the mechanism of sensitivity/tolerance to UV-B radiation. The role of polyamines in the response of a photosynthetic apparatus without LHCII to UV-B is discussed.

P1800. Photosynthetic leaf properties and UV-B protection of cloud forest trees along an altitudinal gradient

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The cloud forest presents a great morphological and structural variation along altitudinal gradients, but information about functional responses is poor. Photosynthetic leaf properties, UV-screening absorbance, and sun and shade leaf characteristics were analyzed in *Vaccinium consanguineum*, *Podocarpus matudae*, *Ticodendron incognitum* and *Drimys granadensis* along their altitudinal distributions. Sun leaves had higher mass and nitrogen per unit area than shade leaves. Chlorophyll (a and b) concentrations, Cha/Chb ratio, and total carotenoids concentrations were similar in shade and sun leaves, but these parameters were affected by the altitudinal gradient. The proportion of nitrogen was measured and partitioned for thylakoids was lower for sun leaves in all species, resulting in different light harvesting capacities between leaves and altitudes. UV-screening absorbance was higher in sun leaves but with no changes related to the altitudinal gradient. Leaf properties were associated with radiation levels along the altitudinal gradient and may reflect CO_2 assimilation rates and protection against UV-B radiation.

P1801. Ultraviolet-B radiation induced changes on growth, photosynthesis and leaf anatomy of broad bean (*Vicia faba* L.)

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The effects of ultraviolet-B (UV-B) radiation on growth, yield, gas exchange rates, pigmentation, soluble sugars, soluble proteins and leaf anatomy of broad bean were investigated. The experiment simulated a 20% stratospheric ozone depletion over Northern Portugal. After 4 months of UV-B treatment, total plant biomass and yield of high UV-B plants were 47 and 56% lower compared to control plants. The decrease of biomass appeared to be the result of changes in morphological, anatomical, physiological and biochemical processes. Enhanced UV-B induced decreases in leaf area, net assimilation rate, photosynthetic and transpiration rate, stomatal conductance, soluble sugars and soluble protein concentration, leaf tissue thickness, as well as in the number and size of root nodules containing the rhizobial symbiont. Pigment analysis of leaf extracts showed no significant effects on accumulation of photosynthetic and UV-B absorbing pigments. Since growing conditions strongly affect plant response to UV-B radiation, further field studies are necessary to assess the interaction between UV-B exposure and environmental variability.

P1802. Susceptibility of Antarctic mosses to UV-B induced DNA damage

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Antarctic vegetation is exposed to elevated levels of UV-B radiation (UVBR) as a result of ozone depletion. Three species of co-occurring Antarctic bryophytes, *Bryum pseudotriquetrum*, *Grimmia antarctici* and *Ceratodon purpureus* were irradiated with artificial UV-B or UVA radiation in either a fully hydrated or desiccated state. Enzyme-linked immunosorbent assays found DNA damage in the form of cyclobutane pyrimidine dimers and 6,4-photoproducts to be specific to UVBR, and DNA damage was significantly higher in the endemic moss, *G. antarctici*, than the other 2 species. In all species, desiccated moss accumulated less damage than hydrated moss. In a season of low ozone depletion, field samples of the 3 species exposed to ambient levels of UV-BR were collected at noon. There was no evidence that DNA damage accumulated in response to field levels of UV-BR. This study demonstrates that desiccation can confer protection from UV-B induced DNA damage in all three species but that *G. antarctici* is potentially more vulnerable to elevated UV-BR than the other 2 species. Continuing ozone depletion events may thus have consequences for the endemic's future competitiveness.

P1803. Effects of solar UV-B exclusion on the physiology of two cultivars of grapevine (*Vitis vinifera* L.) from La Rioja (Spain)

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Plants of two cultivars of *Vitis vinifera* (Tempranillo and Viura) typical of La Rioja (Spain) were exposed for 15 days in the field to two radiation regimes: near ambient radiation, and near ambient radiation 95% depleted in UV-B. The responses of the two grapevine cultivars to the almost complete exclusion of solar UV-B were somewhat different. Viura plants were hardly affected, except for a decrease in methanol-extractable UV-absorbing compounds (MEUVACs) and a less intense action of the xanthophyll cycle. These responses were also found in cv. Tempranillo, together with an increase in the concentrations of chlorophyll and carotenoids and in SPAD values. Thus, solar UV-B seems to cause slight damage in Tempranillo plants, but the rest of the variables (photosynthetic pigment composition, chlorophyll fluorescence) remained unaffected. The damage displayed by cv. Tempranillo as compared to the unaltered cv. Viura could be related to the lower capacity of the former to increase MEUVACs under solar UV-B. In conclusion, both cultivars seem to be adapted to the high solar radiation typical of the Mediterranean climate.

P1804. Protection against a sub-Antarctic summer - photoprotective and UV-B screening pigments of Heard Island vascular plants.

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Two vascular plant species (hair grass *Deschampsia antarctica* and herb *Acaena magellanica*) from sub-Antarctic Heard Island were sampled on 5 days between December 2003 and February 2004. Chlorophyll fluorescence and leaf temperature were determined in the field over midday and photosynthetic tissue samples collected for subsequent analysis of photosynthetic and UV-B screening pigments. *D. antarctica* had low F_v/F_m values coupled with high photoprotective pigment ratios, indicating sustained photoprotection during the study period while *A. magellanica* showed little sign of photoprotection. *Acaena magellanica* contained more than 3 times the amount of UV-B screening pigments than *D. antarctica*. Photoprotective pigment content in both species was responsive to naturally occurring changes in temperature and PAR with *D. antarctica* showing low temperature xanthophyll-mediated photoprotection, whilst *A. magellanica* indicated potential for protection at higher temperatures. Trends in pigments levels and response to temperature and PAR are discussed in relation to photosynthetic acclimation, plant growth habit and survival under a changing climate.

P1805. Impact of elevated UV-B radiation in high altitudes on secondary metabolite profiles in flowering heads of *Arnica montana*

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The influence of intensified UV-B radiation at higher altitudes on the contents of secondary metabolites in flowering heads of *Arnica montana* was assessed. Plants of the cultivar ARBO were grown in nine experimental plots at altitudes between 590 and 2230 m at Mount Patscherkofel near Innsbruck/Austria.

The total contents of sesquiterpenoids and flavonoids were not positively correlated with altitude. However, the proportion of flavonoids with vicinal free hydroxyl groups on ring B to flavonoids lacking this feature significantly increased with elevation. Additionally, the level of phenolic acids also positively correlated with the altitude of the growing site. In particular, amounts of 1-methoxyoxaloyl-3,5-dicaffeoylquinic acid significantly increased at higher sites and samples from the summit region contained 75% more of this compound than samples from valley sites. The UV-B protective and radical scavenging activity of phenolics and their significance for plant life in environments with elevated UV-B radiation are discussed.

P1806. The effect of epibrassinosteroid and different bands of ultraviolet radiation on the pigments content in *Glycine max.L*

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The effect of different bands of UV radiation and epibrassinosteroid treatment on the quantity of chlorophylls, carotenoids, flavonoids and UV absorbing compounds in leaves of *Glycine max* were studied. Study showed in those plants that treated with UV-A the contents of these pigments were not significantly different in comparison to the control. While UV-B and UV-C irradiation caused significant decrease in chlorophylls and carotenoid. Epibrassinosteroid moderated this reduction. The quantity of anthocyanine, flavonoids and UV-absorbing compounds in plants that were treated with UV-B, UV-C and epibrassinosteroid were increased significantly. Therefore we concluded that chlorophylls and carotenoids are sensitive against UV-B and UV-C and application of epibrassinosteroids can protect plants against UV-radiation by increasing protective pigments. Flavonoid, anthocyanine and UV absorbing compounds have high absorption in UV spectrum of light and therefore could prevent penetration of UV to the more sensitive tissues.

P1807. Dynamics of phytohormone and carbohydrate pools in subterranean organs of wintering plants

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During the last 30 years the wintering perennial plants in the central regions of RF are often subjected to severe low temperature diseases. The dynamics of morphological and physiological state of mint rhizomes and tillering nodes of cereals during winter period was studied. The important significance of the maintenance of shoot and root apical meristems in viable state for plant survival was shown. The high activity of carbohydrate and phytohormone metabolism in the subterranean organs of these herbaceous species was revealed. The dynamics of cytokinin / ABA and soluble carbohydrate / starch ratios reflected the thaw phenomena during the winter. The results of evaluation of resistance to winter stresses and snow mold of plastid apparatus for the varieties of perennial wheat and mint will be presented. The selected varieties of this species manifesting a high level of resistance to abiotic and biotic stresses during their life cycle will be characterized. This work was supported by the Ministry of Education and Sciences of RF (project LScSc-1864.2003.4) and by RAS (Program "Fundamental basis of biological resources management").

P1808. Effect of winter desiccation on antioxidant enzymes and anthocyanins in *Vaccinium myrtillus L.*

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Plants face exposure to reactive oxygen species during photosynthesis and cell signaling as well as under environmental stress. Glutathione S-transferases (GSTs), enzymes with multiple cellular functions, catalyse the glutathione-dependent defence reactions and scavenge oxidative stress metabolites by functioning as glutathione peroxidases. Furthermore, GSTs are involved in anthocyanin metabolism, in which they act as carrier proteins. The snow cover crucially protects northern plants from wintertime drought and photo-oxidation in spring. To study the effects of winter desiccation possibly following the predicted climatic warming in the northern boreal zone, bilberry (*Vaccinium myrtillus L.*) plants were exposed to drought and excess irradiation by artificially preventing the formation of a protective snow cover. Control plants wintered under the natural snow. Antioxidant enzyme activities and anthocyanins were determined during winter dormancy, spring, active growth and frost hardening. The winter desiccation induced oxidative state in current-year shoots during summer, which indicates a long-term response to photo-oxidative stress.

P1809. Winter adaptive chances in Magnolias while introduction in Ukraine

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Winter adaptive peculiarities of 10 Magnolia species while introduction in Ukraine was studied. The comparative analysis of

quantitative contents of flavonoids (rutin and quercetin) during autumn-winter months (October-February) shown the majority flavonoids loosed in bark of *M. salicifolia* shoots (75%) and *M. kobus* (56%), the minority - in *M. liliflora* (26%). The content of glycolipids (MGDG) in bark *M. salicifolia* annual shoots was 46 % higher than in shoots of *M. Liliflora*. The shoots of *M. salicifolia* characterized by highest pool of SQDG while the lowest autumn temperature -3°C. In winter months polar lipid components decrease while sterols quantity increase in bark shoots of *M. Liliflora*. The additional investigation of the secretory structure in annual shoots shown the secretory idioblasts accumulation in bark parenchyma. They are characterized by the specific colour of fluorescence from bright-yellow to yellow-green known as fluorescence typical for the natural phenolic and terpenoid fluorochromes. While wintering secretory accumulations which plants use for their physiological needs was observed.

P1810. Effect of polyols on photosynthetic response of foliose lichens to sub-zero temperature

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At sub-zero temperature, cryoprotective compounds help algal lichens to maintain physiological activity. An alga-produced ribitol and hyphal cortex-produced mannitol are main high-energy compounds located in a lichen thallus. We tested hypothesis whether physiological concentrations of ribitol and mannitol provide effective cryoprotection that is detectable at the level of primary photosynthetic processes. From fresh *Umbilicaria hirsuta* and *Lassalia pustulata*, circular segments (1.5 cm²) were cut. Two hours before exposition to low temperature, the segments were treated by 3 different concentrations of ribitol or mannitol under room temperature. In temperature gradient cultivator, the segments were exposed to 3 temperatures (-18, -6, +6 °C) for 3 d at low light. Throughout the experiment, chlorophyll fluorescence parameters (F_v/F_m , Π , NPQ) were measured each 12 h using a kinetic fluorometric CCD camera. For both species, F_v/F_m , Π decreased towards sub-zero temperature and also with length of exposition. Positive effect of polyols on F_v/F_m , Π was apparent only at +6 °C. Species-specific response to polyol concentrations was also apparent.

P1811. Photosynthetic responses of the mangrove, *Avicennia marina* subsp. *australasica* to frost and chilling temperatures during a New Zealand winter

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Avicennia marina subsp. *australasica*, New Zealand's only mangrove, is confined to sheltered coastlines of the northern North Island between Lat. 34° and 38°S. At its range limits, night frosts and chilling temperatures below 5°C are a common occurrence during winter. Although *Avicennia* is mildly frost tolerant, both factors are considered important in determining the southern limit of this species in NZ. Over a 7 month period, including winter, we investigated the course of changes in foliar chlorophyll content and of impacts on the photosynthetic apparatus of *Avicennia* sun and shade and leaves. Marked decreases in the maximum potential of photosystem II (PSII) efficiency, net CO₂ assimilation rates and stomatal conductance were evident in all leaves. The extent of chlorophyll bleaching and reduced PSII efficiency in the sun leaves was consistent with photoinhibitory damage (chlorophyll a + b content declined to 76.3% and PSII efficiency to 43.7% of start values). It was concluded that physiological performance in *Avicennia* is significantly depressed by cold winter temperatures, and that critical damage can be sustained at temperatures above freezing level.

P1812. Effects of plant growth and photo-oxidative stress on leaf senescence of *Quercus crispula*

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To investigate how plant growth and photo-oxidative stress affect leaf senescence, *Quercus crispula* seedlings were grown from seeds in growth chambers in combination of two light intensities and two temperatures. The photosynthetic rate, F_v/F_m , amount of xanthophylls and dry weight of the seedlings were measured for indices of leaf senescence, photo-oxidative stress and plant

growth. From the early stage of leaf senescence, F_v/F_m value was lower and the amount of xanthophylls and de-epoxidation rate were higher in plants grown under low temperature with high light than those in other growth conditions. The photosynthetic rate was higher in the early stage of senescence but decreased to a great extent in plants grown under high temperature with high light, with a considerable increase in plant dry weight. It was suggested that leaf senescence was affected by photo-oxidative stress under low temperature with high light and by plant growth under high temperature with high light.

P1813. Effect of Different Air and Root-zone Temperatures on Nitrogen Fixation and Nodulation of Annual Medics of Iran

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Three annual medic cultivars (*Medicago polymorpha* cv. Santiago, *Medicago radiata* cv. Radiata, and *Medicago rigidula* cv. Rigidula), were evaluated for symbiosis traits under three levels of day/night air temperatures (DNAT, 15/10, 20/15, and 25/20 ±0.2°C) and four root-zone temperatures (RZT, 5, 10, 15, and 20±0.2°C). The result showed that low root zone temperature (RZT) and day/night air temperature (DNAT) had a severely reduction effect on root length, root dry matter and nodule formation. Plants did not produce any nodule in the root at 5°C RZT and all DNATs. The plants grown at RZT ranging from 10 to 20°C were found to fix some nitrogen. The 10°C RZT seem to be a thermal critical point for *Medicago* spp. nodulation and N₂ fixation. DNAT also had the same effect on plant nitrogen fixation. *M. rigidula* performs well in nodule formation and nitrogen fixation at low RZT. Therefore *M. rigidula* is probably the most promising for the production of herbage at low temperature. *M. polymorpha* was found to have a significant performance in moderate temperatures but was also found to be the most widespread species and thus may be of high priority in cultivar development.

P1814. Antifreeze Proteins Modify the Freezing Process in Planta

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The goal of this study was to determine how AFPs reduce freezing injury in cold-acclimated winter rye plants. Antifreeze activity was present in guttate obtained from cold-acclimated winter rye leaves, indicating that the AFPs are soluble in the apoplast and can bind to the surface of intercellular ice. As shown by infrared video thermography, AFPs had no effect on supercooling of the leaves, but AFPs did lower the freezing temperature of the leaves by 0.3 to 1.2°C when an ice nucleator was present. In vitro studies showed that apoplastic proteins extracted from cold-acclimated winter rye leaves inhibited the recrystallization of ice and also slowed the rate of migration of ice through solution-saturated filter paper. We found that lactate dehydrogenase activity was higher after freezing in the presence of AFPs, but the same effect was obtained by adding bovine serum albumin, and that AFPs had no effect on unstacked thylakoid volume after freezing and thawing. We conclude that rye AFPs have no cryoprotective activity, rather they interact directly with ice *in planta* and reduce freezing injury by slowing the propagation and recrystallization of ice in the tissues.

P1815. Comparing photosynthetic, photoprotective and water relations parameters in winter-red and green phenotypes of *Cistus creticus*

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The foliage of a considerable number of individuals in *Cistus creticus* turns red during winter after accumulation of anthocyanins. In contrast, other individuals occupying the same habitat remain green throughout the year. When green, the leaves of both phenotypes have similar photosynthetic pigment levels, water potential and stomatal conductance, but PSII effective photochemical yield and corresponding electron transport rates are slightly lower in winter-red phenotypes. When redness appears in winter, red phenotypes display lower xanthophyll cycle pools and lower stomatal conductances, but similar water potentials, PSII

effective photochemical yields and electron transport rates. Maximum PSII yield is high and similar through out the year. We suggest that subtle intraspecies differences in photosynthetic rates may become critical under low temperatures, leading to the development of an anthocyanic, photoprotective screen in the most sensitive individuals.

P1816. Regulation of Chilling on the Expression of Chloroplast ATP Synthase and its Relation to Photosynthesis in Rice

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The objective of this study is to investigate the regulation of chilling on chloroplast ATP synthase and its relation to photosynthesis in rice. Photosynthetic rate of rice was significantly reduced under low temperature (8 °C), but chilling-tolerant cultivar remained at higher level than chilling-susceptible cultivar. Chloroplast ATP synthase activity in chilling-tolerant cultivar was not affected, while that in chilling-susceptible cultivar decreased under chilling. *atpH-atpF-atpA* transcripts were significantly reduced at low temperature after treated for 1 to 5 d. However, *atpB-atpE* transcripts showed different patterns. The large transcripts with strong signal were not affected by chilling, while the small and weak transcripts were reduced by chilling. *rbcL* transcript was not affected by chilling. Western blot analysis revealed that the protein contents of subunit and III and Rubisco large subunit were not affected under chilling. ABA, sucrose, H₂O₂, paraquat, Ca²⁺, EGTA, LaCl₃ had no influence on the expression. There is no difference of all above genes expression between chilling tolerant cultivar and susceptible one.

P1817. Low-temperature response of two clones of Eucalyptus globulus L.

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The low cold resistance of *Eucalyptus globulus* L. is the main factor that affects its growth and restricts the cultivation area. During cold acclimation numerous physiological changes are induced that allow plants to develop efficient tolerant mechanisms needed for winter survival. The main objective of this work was to evaluate cold resistance and capacity for cold acclimation of two highly productive *E. globulus* clone (ST51 and CN5) in order to understand the strategies that may provide protection at cellular level. Metabolic response to supoptimal temperatures was studied by comparing the effect of cold treatment on membrane integrity and lipid composition, activity of antioxidant enzymes, proteins, pigments (chlorophyll, b-carotene, antheroxanthin) and soluble and insoluble sugars content in leaves and roots of eucalyptus plants in control conditions (22/16°C, day/night) and after a gradual temperature decrease period to 9/4°C, day/night.

Cold acclimation of two *E. globulus* clones was achieved owing to modification in growth, membrane fluidity, activity of antioxidant enzymes, sugars content and correlate with response to water deficit.

P1818. Changes of Carbohydrate Concentration in Cuttings of Deciduous Azalea Cultivar 'Polarzvaigzne'

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This study has focused on changes of carbohydrate contents during rooting of cuttings and on stock plant forcing in a greenhouse during the spring as a management of the stock plants.

The concentrations of reducing sugars (RS), sucrose, total soluble sugars (TSS = RS + sucrose), and starch were determined in the leaves and the stem bases of cuttings.

Overall, the patterns of carbohydrate changes were similar in forced and control cuttings. The concentrations of RS, TSS, and starch increased essentially in the leaves and the stem base of cuttings after severance. The concentration of sucrose generally fluctuated over time. In the leaves the sucrose concentration was lower than the RS concentration throughout the rooting process. After root emergence the RS, sucrose, and starch concentrations

decreased initially in the stem base and later in the leaves of cuttings.

The results demonstrated that the stock plant forcing did not significantly affect the rooting process and the starch concentration was better related to the rooting of cuttings than the TSS concentration.

This study was supported by the European Social Fund.

P1819. Evidence a new glycoside ester from Cochlearia genus.

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This work reports the procedure for extraction, purification, chemical characterisation and quantification of a new glycoside in halophytic plants. This new natural compound, 1-O-(2-methylbutyryl)-D-glucopyranoside, was isolated from the aerial parts of *Cochlearia* species, collected along coastal sites of Brittany. An HPLC method for the isolation of this compound is proposed. The crude extract of *Cochlearia* was purified using an Aminex HPX 87C column eluted with deionised water at 85°C. The structure of the product was established mainly on the basis of spectroscopic NMR data (¹H, ¹³C, COSY, TOCSY, HMQC, HMBC, J-MOD). A simple, sensitive and selective HPLC method with UV detection (226 nm) was developed for quantification of 1-O-(2-methylbutyryl)-D-glucopyranoside. The occurrence and abundance of this glucoconjugate in *Cochlearia* genus and in other halophytic species of Brassicaceae is of interest concerning its chemotaxonomical and ecophysiological potential.

P1820. Sugar components of floral nectar in quince (Cydonia oblonga Mill.) cultivars

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The sugar composition of the floral nectar was studied in 22 mainly Hungarian local quince cultivars in 2004. The material for investigation was taken from the quince genebank of the Research and Extension Centre for Fruitgrowing, Újfehértó, Hungary. Sugars were analysed by thin layer chromatography.

The three investigated nectar sugar components were glucose, fructose and sucrose. Sucrose was present in the greatest amount in the nectar of all investigated quince cultivars. Its quantity varied from 47 (cv. 'Váli') to 75 % (cv. 'Késői Kiskunfélegyházi'). The amount of the total nectar sugars was the lowest (159 mg/ml) in cv. 'Pear-shaped Noszvaji' and the highest (552 mg/ml) in cv. 'Kúti'. The nectar of most cultivars contained more fructose than glucose. According to the Baker-quotient (S/F+G), most of the cultivars were found to have sucrose dominant nectar. However, the nectar of the cultivars 'Mezőkövesdi', 'Apple-shaped Dunabogdány' and 'Váli' was sucrose-rich. The above results are important from the viewpoint of pollination biology, apiculture and defence against fire blight.

P1821. Mobilization of raffinose oligosaccharides during seed germination

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Raffinose family oligosaccharides (RFOs) accumulate during seed maturation and are rapidly mobilized at the early stages of germination by alpha-galactosidases. We investigated the role of RFOs during germination by treating seeds of *Pisum sativum* with a potent alpha-galactosidase inhibitor (DGJ). The germination rate of seed treated with DGJ was about 50% lower than that of controls, providing evidence that RFOs act as an important energy source during the early stages of germination.

During seed maturation alpha-galactosidases are deposited in protein storage vacuoles (PSVs), which merge to greater vacuoles during seed germination. Separation of PSVs from the cytoplasm of imbibed seeds showed that RFOs are mainly stored in the cytoplasm. By using a fluorescent substrate and confocal laser scanning microscopy, we could prove that alpha-galactosidases were active in PSVs during early stages of germination. Thus, the breakdown of RFOs in germinating seeds may involve an import of RFOs into PSVs, while the resulting sucrose and galactose must be transported back to the cytoplasm for further metabolism.

P1822. Effects of water deficit on chlorophyll content, water potential and stomatal properties of Anise (*Pimpinella anisum* L.).

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For studying the effects of soil available water (SAW) on chlorophyll content, water potential and stomatal properties of Anise (*Pimpinella anisum* L.), a greenhouse experiment with five water supply treatments (20, 40, 60, 80 and 100 % of SAW) was carried out in a randomized complete block design (RCBD) with four replications in the agricultural research of Tabriz University. The results showed that relative water content (RWC) and water potential of Anise in stem elongation and umbel appearance stages were significantly reduced when water supply decreased from 80 % of SAW. Also, there was a linear relationship between water supply and chlorophyll a and b content of leaves. Water deficit in stem elongation stage increased stomatal density, but in such conditions, the length of stomatal pores was reduced. However, water deficit had no significant effect on the width of stomatal pores. It may be concluded that low soil available water (less than 80%) affects the evaluated ecophysiological properties of Anise and therefore the growth, yield and essential oil production of this medicinal plant.

P1823. Stomatal biophysics: What has changed in 400 million years?

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Stomatal guard cells are the most complex biomechanical plant structures, yet, apart from a distinctive modification in grasses, have remained relatively unchanged in form since they appeared in the earliest vascular plants some 400 MY ago. Since the first prototype, plants have never improved on the "bending guard cell pair" as a mechanical solution for regulating the passage of gas molecules across the epidermis. However, within this design constraint there is considerable scope for altering guard cell mechanics such that the motor forces into which environmental signals are translated achieve optimal pore width for any given series of environmental modulations. Innovations in cell microprobe techniques, cell imaging and biochemical manipulation are providing new insights into the dynamic properties of guard cells and how these properties change in different environments. In support of plant diversification throughout an ever changing global climate, the stomate has undergone several key biophysical adaptations.

P1824. Where does the signal for guard cell differentiation come from?

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Stomata (*st*) are non-randomly distributed on the leaf surface. The underlying mechanism is attributed to two genes/signalling proteins inducing asymmetrical division of meristemoid cell. We show that the diffusion rate (DR) of water can be a factor affecting the epidermal cell fate.

Lepidium sativum and *Zea mays* were grown from seeds in mesocosm atmosphere of (i) helium with additional 21 % O₂ and 350 μmol mol⁻¹ CO₂ (He/Ox) or (ii) artificial air (the same mixture with He substituted by N₂, Ni/Ox). DR of H₂O and CO₂ is 2.6 times faster in He/Ox than in Ni/Ox. Humidity was controlled. Number and distribution of *st* were evaluated on imprints of cotyledons. Leaf anatomy gas exchange and ¹³C discrimination (¹³C) were investigated.

St of *L. s.* from He/Ox were grouped into clusters of 2-4 while those from Ni/Ox were distributed regularly. Rising humidity enhanced this effect. Distribution of *st* in *Z. m.* was not affected. Leaf anatomy and ¹³C indicated faster diffusion in He/Ox than in Ni/Ox leaves. The results show different signalling in differentiation of *st* in monocots and dicots and significance of DR of water vapour.

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P1825. Reactive oxygen species (ROS) production during bicarbonate induced stomatal closure in abaxial epidermis of *Pisum sativum*

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The guard cells respond strongly to signals, such as light, CO₂ or plant hormones. The presence of 2 mM bicarbonate in the incubation medium induced stomatal closure in abaxial epidermis of *Pisum sativum* L and such stomatal closure was partly prevented by exogenous catalase (CAT) and diphenylene iodonium (DPI). Exposure to 2 mM bicarbonate elevated the levels of reactive oxygen species (ROS) production in guard cells, as indicated by the fluorescent probe dichlorofluorescein diacetate (H₂DCF-DA). The ROS production reached maximum by 5 min on exposure to bicarbonate. The bicarbonate induced increase in ROS production of guard cells was suppressed by the application of CAT or DPI. The results suggest that H₂O₂ is an important secondary messenger during bicarbonate induced stomatal closure in abaxial epidermis of *Pisum sativum*.

P1826. Ionic basis of light signalling in guard cells

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Stomata of most plants open in the light, thereby enabling uptake of CO₂ for photosynthesis. Two light signalling pathways operate in parallel in guard cells, a blue light specific pathway and one dependent on photosynthetic active radiation (PAR). The blue light pathway is initiated by two phototropin receptors and leads to activation plasma membrane H⁺-ATPases, while the PAR pathway involves changes in the intercellular CO₂ concentration and inhibits anion channels. In search for a possible interaction of both pathways, we conducted experiments with albino plants. Stomata of albino plants still opened in blue light, but not in red light, showing these stomata lacked the response to PAR. Recordings with intracellular micro-electrodes, on guard cells located in intact plants, revealed that blue light generated outward H⁺-ATPase currents of 37 pA in albino guard cells and 51 pA control cells. Apparently, this blue light-response is independent of a direct effect of PAR on guard cells.

P1827. Primary production and nutrient accumulation in two saltmarsh species, *Atriplex portulacoides* L. and *Limoniastrum monopetalum* L., in Southern Portugal

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Seasonal patterns of above and belowground biomass, net primary production and nutrient accumulation were assessed in *Atriplex portulacoides* and *Limoniastrum monopetalum* in Castro Marim saltmarsh, Portugal. Sampling was conducted in five periods during 2001/2002 (autumn, winter, spring, summer and autumn).

Mean live biomass was 1808 g m⁻² for *L. monopetalum* and 653 g m⁻² for *A. portulacoides*. Peak living biomass, in spring for both species, was three times higher in the former, 3500 g m⁻², than in the latter, 1076 g m⁻². However, while green leaves and stems attained the highest proportion in *A. portulacoides*, woody stems were the main component in *L. monopetalum*. Belowground biomass of *L. monopetalum* was 1,7 times higher than that of *A. portulacoides*. In spite of this *A. portulacoides* showed higher root/shoot ratio along the year. Annual net primary production was 1029 g m⁻², for *A. portulacoides* and 4444 g m⁻², for *L. monopetalum*.

Nutrient concentrations showed similar patterns throughout the year. *A. portulacoides* showed lower values of N, Mg and Ca, and higher values of K, than *L. monopetalum*, while the values of Mn and P were similar.

P1828. Response of the non-CAM succulent *Augea capensis* (Zygophyllaceae) from the southern Namib Desert, to drought stress

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Plants of *Augea capensis* Thunb. were transferred from their natural habitat in the Namib desert (Namibia) to pots and subjected to drought by withholding water for 20 days. To assess drought stress effects on the physiology of the plants, chlorophyll a fluorescence and photosynthetic gas exchange were measured. An anatomical investigation was also conducted and ultra structural changes assessed. Following drought stress, the recovery potential of the plants was monitored.

Chlorophyll a fluorescence kinetics indicated a loss in photosystem II electron transport capacity during drought stress. The observed decrease in carboxylation efficiency and CO₂ saturated rate of photosynthesis provides additional support of drought-induced mesophyll limitation of photosynthesis. After 20 days of drought, cell organelles showed typical signs of drought stress, but were still intact. Large numbers of lipids found in leaves of control plants were absent in drought-stressed plants. Following re-watering, a gradual recovery of photosynthetic capacity was observed.

P1829. The study of protein content and gel electrophoresis in mycorrhizal symbiosis *Pisum sativum* L. with *Glomus etunicatum*

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In this research we studied the effects of mycorrhization on protein content and gel electrophoresis of roots, leaves, and seeds in *Pisum sativum*. After reproduction of *Glomus* with *Sorghum spp.* and *Zea mays* that are susceptible to mycorrhization, *Pisum sativum* were cultivated in medium containing inoculum of *Glomus*. Results of assay the proteins with Bradford methods show that at the eighth weeks, protein content of roots, leaves and seeds of mycorrhizal plant in compare with the control plants are higher.

Also the separation of electrophoretic bands of leaves, roots and seeds proteins in SDS-PAGE gels have shown the new bands in mycorrhizal samples; for roots in three main regions, 18.4 to 25 kDa and second between 35 to 45 kDa, and the other between 66.2 to 116 kDa, for leaves in two main regions, the first 14.4 to 25, and the other between 25 to 45 kDa, for seeds in four main regions, 14.4 to 18.4 kDa, 18.4 to 25 kDa, 35 to 45 kDa and 45-66.2 kDa.

P1830. Some Ecophysiological Properties of Tree Seedlings

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Drought, extreme temperatures, air pollution and other stress factors, lead to significant modification in ecosystems and affect the reduction of the number of tree species that can be grown.

The investigation was carried out on the seedlings of *Picea abies* Karst., the main species of coniferous forests, *Fagus moesiaca* Domin. Maly. Czeczott., the most widely distributed species in Serbia, *Betula pendula* Roth., a fast growing, pioneer species with very wide ecological adaptability and *Acer heldreichii* Orph., an endemic and relic of Balkan Peninsula.

Physiological parameters, water potential, transpiration intensity and the content of biogenous elements (N, P, K, Ca, Mg) in leaves were measured as well as growth parameters.

Variability of ecophysiological characteristics between individuals of the same species on the same ecological site, show the presence of genetic specificity. The most xeromorphic leaves were those of beech from Mt. Kopaonik and the least of leaves of Balkan maple.

The application of the researched ecophysiological criteria can accelerate and develop the selection procedures tree species for the needs of seedling production.

P1831. Preliminary data about heavy metals accumulation in *Xanthoria parietina* from Madrid Province

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Numerous studies have documented the ability of lichen to accumulate trace elements from the environment. This characteristic has been used to indicate exposure to trace elements along highways, near smelters, in industrial areas, etc. Thanks to their much higher accumulation factors, related to

characteristic thallus structure and longer life, lichens are more advantageous bioindicators than plants.

Several studies have demonstrated the presence of heavy metals in particles trapped in the cell wall, though the damage in the thallus seems to occur as a consequence of uptake of heavy metals into cells.

Xanthoria parietina is a pollution tolerant lichen, collected of different areas from Madrid Province. These samples were analyzed by conventional scanning electron microscopy (SEM) equipped with a LINK eXL X-ray dispersion detector for microanalysis of the occurrence of heavy metal particles.

The analysis of the samples indicates the presence of Ti, Fe, Si, Cr and Al as main components of the elements retained in the cell wall. It has been discussed if these heavy metals can be contaminants or particles belonging to the substrate in which the lichens live.

P1832. Diversity in functionally important traits of promiscuous group of *Sesbania-rhizobia* and their colonization potential to ecologically diverse habitats of a semi-arid region of India

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Diversity in functional traits of 28 root- and stem-nodulating bacterial isolates of *Sesbania sesban*, *S. aegyptica* and *S. rostrata* inhabiting 6 ecologically distinct sites of semi-arid Delhi region was analysed. The isolates were highly promiscuous among *Sesbania spp.* and formed morphologically similar nodules differing in symbiotic properties. 16S rDNA sequence analyses revealed that root nodulating isolates belong to *Sinorhizobium saheli*, *S. meliloti* and *Rhizobium huautlense* whereas *Azorhizobium caulinodans* nodulate the stem. *Sinorhizobium spp.* dominate as microsymbiont partner of *Sesbania* in Delhi region. Grouping of isolates into types / clusters based on LPS and NPC-PCR analyses suggest its significance in circumscription of the taxa. However, subtypes and subclusters showed their sorting across the ecological gradients. Enormous diversity in LPS profiles and high specificity of phages might be result of environmental selection pressures operating in ecologically distinct habitats. Effective promiscuity of *Sesbania* and variations in functionally important traits of rhizobia contribute to wide ecological amplitude of the legume host.

P1833. Effects of vesicular arbuscular mycorrhiza (*Glomus etunicatum*) on the chlorophyll content, nodulation and growth parameters in *Pisum sativum* L.

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Endomycorrhizal symbiosis (vesicular arbuscular mycorrhizae) increases the rate of growth and uptake of some elements in plants. In this research, the effects of pea plants mycorrhization on the growth parameters, chlorophyll content and nodulation investigated. In this purpose, *Glomus etunicatum* separated of infected soils and pure. Pea plants were cultivated in a medium containing inoculum of *Glomus*. The study of symbiosis and growth parameters performed in sixth, eighth and tenth weeks after inoculum. At this time the number of nodules counted and its size in the control plants roots and mycorrhizal plants roots measured. In compare with the control plants, growth parameters such as leaf numbers, leaf area, pods length, flower numbers, number of seeds, dry weight of roots, leaves and seeds, chlorophyll content in mycorrhizal plants increased. As well as the number of nodules and its size was higher than the control plants.

P1834. Influence of sulphur nutrition on cadmium induced changes in photosynthetic rate and its related variables in mungbean

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Mungbean (*Vigna radiata* L. Wilczek) is a potential crop in the Indian sub-continent due to its ready market, nitrogen fixation capability, early maturity and ability to fit well into a crop rotation programme. In the present investigation, an attempt was made to work out the ameliorative effect of sulphur in cadmium toxicity of mungbean. Mungbean was grown in earthen pots at two levels of

sulfur (S) viz. 0 and 40 mg S/kg soil and four levels of cadmium (Cd) viz. 0, 25, 50 and 100-mg Cd/kg soil. All the cadmium levels, when applied alone, severely reduced the photosynthetic rate and its related variables; the extent of reduction increased with increase in Cd level. A concomitant supply of sulphur (i.e. S + Cd) significantly ameliorated the toxic effect of cadmium and improved the photosynthetic efficiency and the related variables. These findings demonstrate the nutritional importance of sulphur in plants against the cadmium toxicity.

P1835. Antioxidant response of BY-2 *Nicotiana tabacum* cells to cadmium stress

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Cadmium can induce the production of reactive oxygen species (ROS) and can cause serious damage to cellular metabolism. Antioxidant enzymes are capable of scavenging heavy metals induced ROS. We have observed alterations in SOD, CAT and peroxidases activities, but a significant increase in GR activity in tobacco BY-2 cells exposed to cadmium (Cd). The results suggest that in BY-2 cells the main defence system to Cd stress is variable during the heavy metal exposure time. The synthesis of GSH, which is used in the synthesis of phytochelatins and substrate for GST is evident during the onset of the stress with CAT and peroxidases taking over the antioxidative responses once the stress becomes severe. The study of such responses may allow the evaluation levels of tolerance, specificity of the response to levels of pollution in the environment. These data may be useful in breeding programs to select tolerant plants and be used in phytoremediation, by reducing the amount of heavy metals in contaminated soils. Financial support by FAPESP, CAPES and CNPq.

P1836. Vegetation studies on a sediment contaminated with chromium (1998-2003)

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In-situ phytostabilization has special significance since it can reduce the dangers associated with the potential toxicity of bioavailable heavy metals. Since vegetation forms a green cover the approach is environmentally attractive and cost-effective.

We present results on the floristic composition, vegetation pattern and the chromium content of the sediment and plants in the units of an abandoned secondary sedimentation pond system of a leather company in Kunszentmárton (Hungary)

In 1998 42 plant species were found in this area, while in 2001 46 ones were determined. Most of these plants found were belonged to the families Compositae and Graminae. In 1998 the 80 % of the area was covered by plants the 80 % of which were five species: *Phragmites australis*, *Bolboschoenus maritimus*, *Echinochloa crus-galli*, *Typha angustifolia*, *Atriplex hastata* and *Aster sedifolius*. Most species found in the area were competitors and native weed species.

P1837. Effects of heavy metal pollution on higher plants, and investigation their availability in the view of phytoremediation

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Phytoremediation, removal and disposal of environmental pollutants with plants utilizes the natural ability of plants to uptake and accumulate different substances in their tissues during their mineral nutrition. Some species can remove large quantities of heavy metals from their environment.

Different plant species were used in our experiments: Indian mustard (*Brassica juncea* L. Czern.), broadleaf cattail (*Typha latifolia* L.), and sunflower (*Helianthus annuus* L.).

Hydroponically grown plants were treated with 100 µM zinc, cadmium or copper for one week. After the treatment metal ion accumulation in the shoot and the root by atomic absorption spectroscopy and chlorophyll a and b levels were measured. Plants accumulated metal ions preferentially in their roots. Copper treatment had a particularly drastic effect on the root system. Treatments caused a decline in chlorophyll levels and inhibited the growth of plants.

The role of antioxidant enzymes, such as catalase, peroxidase, superoxide dismutase, glutathione reductase, and glutathione-S-transferase were also investigated.

P1838. Ecological & biochemical peculiarities of plant resistance to oxide and lead stress

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The quantity of available heavy metals in soil is increased greatly in industrial areas and being uptaking by plants they induce toxic and adaptive responses. One of the first unspecific reactions in development of the general adaptive syndrome is an activation of lipid peroxidation processes. Therefore experimental plants *Triticum aestivum* L. were sprayed at tillering stage by Pb(NO₃)₂ or H₂O₂ solution twice. Data obtained showed that lipid peroxidation was activated in both variant of experiment. Lead treatment caused glycolipid component increase, especially monogalactosyldiacylglycerol, whereas H₂O₂ caused it content decrease accompanied by sulphoquinovosyldiacylglycerol accumulation. We consider their role in adaptation processes to be connected with homeostasis fluidity of photosynthetic membranes and general adaptive reaction of agrophytocenose to anthropogenic pollution.

P1839. Ni, Pb, Zn concentrations, peroxidase activity and chlorophyll content of some trees in Istanbul (Turkey)

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In this investigation, concentrations of Ni, Pb and Zn have been measured in soil and in the *Acer*, *Aesculus*, *Populus*, and *Robinia* leaves that were collected from the urban sites of Istanbul (Turkey). Peroxidase activity (POD) and chlorophyll content (Chl) were determined to observe some ecophysiological changes in leaves. Concentrations of the elements, POD and Chl showed variations depending on the tree species and sites. Highest Zn (593 mgkg⁻¹dw) was found in *Populus*; highest Ni (5,3 mgkg⁻¹dw) and Pb (29 mgkg⁻¹dw) were found in *Robinia* and *Aesculus*, respectively. In soil samples, highest Ni, Pb and Zn concentrations were determined as 45, 1121 and 529 mgkg⁻¹dw. Lowest Chl (0,68 mgg⁻¹fw) and highest POD (840 Ag⁻¹fwmin) were detected in *Acer*. We have defined a significant positive correlation between Pb-POD in *Populus* (<0,05), and between Pb-Zn in *Acer* and *Aesculus* (<0,01). Significant negative correlations were observed between Pb-Chl and Chl-POD in *Aesculus*. Investigated species are considered to have different tolerance levels to heavy metals. The data obtained shows that, Chl and POD may be used as heavy metal stress biomarkers in the urban trees.

P1840. Regulation of antioxidant activity in lead treatment Soya plants by sulphur compounds

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Soya plants were grown with or without sulphur in nutrition medium. Oxidative stress was induced by treatment of 14 day Soya shoots with 0,5mM Pb(NO₃)₂. MDA content increased in the plants of both variants on the six hour of Pb stressor influence. SOD activity was found to be depressed by 12% in plants grown without sulphur fertilization but it returned back to control in 24 hours. On the contrary, plants grown with sulphur addition had SOD activity increased by 17% in six hours and by 22% in 24 hours. Free thiol content decreased in six hour in treated plants of the two variant. Plants grown without sulphur showed tendency to decrease of free and protein thiol content in 24 and 48 hour. Content of protein thiols in Soya plants grown with sulphur in nutrition medium was much higher than in plants grown without sulphur. Protein thiol content decreased in treated plants in six hour of Pb stressor action, but became stabilized in 24 and 48 hour. The role of sulphur compounds is Soya plants resistance to oxidative stress caused Pb(NO₃)₂ treatment discussed.

P1841. Copper mine waste and Cu, Fe, Ni, Pb accumulation in some plant species

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CMC (Cyprus Mining Corporation) area is located in the northwest coast of Cyprus (Lefke, Gemikonagi). CMC operated copper mine until 1974, but the spread out nature of the facility left widespread piles of tailing deposits of hazardous waste and heavy metal accumulation caused soil and water pollution. In this study, we aimed to determine tolerant plant species of this area and Cu, Fe, Ni, Pb concentrations in plants and soil samples. *Acacia cyanophylla*, *Asparagus* sp., *Atriplex semibaccata*, *Juncus* sp., *Phagnalon rupestre*, *Pistacia terebinthus*, *Polygonum equisetiforme*, *Tragopogon sinuatus* were detected as naturally growing plant species around a selected copper flotation tailing pond. Accumulations of Cu (74,9-282,6 mgkg⁻¹dw) and Fe (674,2-767,4 mgkg⁻¹dw) were found high in the above ground parts of *Acacia*, *Atriplex* and *Juncus*. Highest soil concentrations were determined as 891,1 mgkg⁻¹dw Cu and 1712,5 mgkg⁻¹dw Fe, respectively. Ni and Pb concentrations were found quite low. We assume that *Atriplex*, *Acacia* and *Juncus* may be thought as tolerant and bioindicator species, which may also be used for phytoremediation to recover the contaminated CMC area.

P1842. The protective effects of gibberellin and ascorbic acid against nickel-induced oxidative damage in soybean plants

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The interactive effects of nickel (Ni) and ascorbic acid (AsA) and gibberellin (GA3) on soybean plants (*Glycine max* L. cv. Union x Elf) were examined. One - week - old hydroponically - grown seedlings were exposed to NiCl₂ · 6H₂O (0.5 mM), with or without AsA (1 mM) or GA3 (0.05 mM) or AsA (1 mM) + GA3 (0.05 mM), for 5 days. Ni toxicity symptoms, such as formation of reddish - brown mottled spots on the leaf blade, observed in Ni - treated plants. Interestingly, with application of GA3 + AsA, this symptoms did not appear in Ni - stressed plants. Ni suppressed biomass production and reduced chlorophyll content . An enhanced level of malondialdehyde and changes in the activities of catalase (CAT) and guaiacol peroxidase (GPX) indicated that Ni caused oxidative stresses. The Ni -treated plants that exposed GA3 + AsA exhibited much better growth. Interaction of AsA and GA3, prevented the decrease in chlorophyll content and lipid peroxidation as well as increasing the activities of CAT and GPX. This results suggest that GA3 + AsA causes to reduce the negative effects of Ni damage in *G. max* L. , but more research is needed to elucidate the precise interactive role they played as antioxidants.

P1843. Changes in antioxidant enzyme activities in *Eichhornia crassipes* under heavy metal stress

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Plantlets of *Eichhornia crassipes* were exposed to various concentrations (0, 0.1, 0.3, 0.5, 1.0, 3.0 and 5.0 mM) of 8 heavy metals (Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn) hydroponically for 21 days, and spectrometric assays for total activity of catalase, peroxidase, polyphenol oxidase and superoxide dismutase in the leaves were studied. Plants were harvested at regular intervals of 1 week and prepared for the enzyme assays. At each time-point data referred to metal treated plants were compared to data of untreated ones (control). There was no significant difference in enzyme activity of the control and those treated with 0.1mM of the heavy metals studied (except Hg). Generally, the antioxidant activities increased with increase in metal concentration and exposure duration. The authors concluded that heavy metals induced oxidative stress and responses of antioxidant enzymes were proportional to the duration of exposure and metal concentrations.

P1844. Cu,Zn-SOD in *Chlamydomonas gloeogama* and Specific Detection of Its Gene by a PCR-Based Method.

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This work demonstrates the first evidence for Cu,Zn-SOD in *Chlamydomonas gloeogama*. The studies were conducted at the protein and DNA levels. With modified native PAGE and activity staining in the presence of H₂O₂ or H₂O₂ plus KCN, two novel Cu,Zn-SOD isozymes were disclosed among Fe-SOD isozymes. Genomic DNA of *C. gloeogama* was amplified by using primers

designed from the highly conserved region of tomato Cu,Zn-SOD cDNA. PCR at various Mg²⁺ concentrations showed that the 200-bp fragment should be the specific PCR product. The primers were also used in amplification of Cu,Zn-SOD gene fragments from tomato genomic DNA obtaining 700-bp products. For probe preparation, DIG-labeling on the 700-bp products was achieved by PCR method. The 200-bp amplified fragment of the algal DNA stringently hybridized to the DIG-labeled probe, thus, demonstrating homologous sequences. On the DNA homology rationale, CuZn-SOD gene was figured out in *C. gloeogama*.

P1845. Water Use in Tank and Atmospheric Bromeliads of a Seasonally Dry Forest in Mexico

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We analyze traits that allow the coexistence of different epiphytic life forms with contrasting strategies for resource acquisition and morphologies. Epiphytic members of the Bromeliaceae have a vast radiation of morphologies that represent two contrasting ecological strategies, tank and atmospheric. These life forms coexist in a wide range of environments, even though physiological differences and morphological differences have been described. We characterized a community from a seasonally dry tropical forest that has a prolonged 8 month drought, during which dew is the only source of water, and measured their seasonal photosynthetic performance. We found that the tank species have most of their photosynthetic activity during the rainy season; our data suggests that these species can intercept dew efficiently to allow their survival during the prolonged drought, counterbalancing their high water loss, compared to the atmospheric. The more drought enduring atmospheric species have most of their activity during the beginning of the dry season, when the dew constitutes their main water source.

P1846. Vertical distribution, composition and diversity of vascular epiphytes along a spatial gradient in Bogotá city, Colombia

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Studies about epiphytes diversity and distribution have been made in forests, but there are just a few made in cities. We analyzed how vertical distribution, composition and diversity of vascular epiphytes change along an East-West spatial gradient of 4 Km in Bogotá, between two mountain chains. The gradient was divided into 10 transects of 400 m long, with 3 replications (total: 40 transects). The phorophytes were subdivided in 5 vertical zones. We found 633 epiphytes individuals of 4 families (Bromeliaceae, Polypodiaceae, Acanthaceae and Araliaceae) and 9 species, growing on 175 phorophytes of 10 species. The most abundant species were *Pleopeltis macrocarpa* (55.45%) followed by *Tillandsia incarnata* (36.33%). There were differences in the vertical distribution between families. Bromeliaceae prefers the medium and high zones of the canopies (2nd, 3rd and 4th zone), whereas the other families grow below those (1st, 2nd and just a few in the 3rd zone). There is a notable increase of diversity and richness towards the East. We explain this because the mountains in the East could represent a continent for dispersion of seeds.

P1847. Strongly and weakly expressed crassulacean acid metabolism in orchids of Panama

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We investigated the occurrence of crassulacean acid metabolism (CAM) in 200 native orchid species from Panama and 14 non-native species using carbon isotopic composition (¹³C) and compared these values with nocturnal acid accumulation measured by titration in 173 species. Foliar ¹³C showed a bimodal distribution with the majority of species exhibiting values around -28‰ (typically associated with the C₃ pathway), or around -15‰ (strong CAM). Even though thick leaves were related to ¹³C values in the CAM range, some thin-leaved orchids were capable of CAM photosynthesis when titratable acidity measurements were performed. With the majority of species in this study being epiphytes, we found that many species within the C₃ isotopic range were capable of significant acid accumulation at night. Of 128 species with ¹³C more negative than -22‰, 42 species showed nocturnal acid accumulation per unit fresh mass characteristic of

weakly expressed CAM. These data suggest that among CAM orchids, there may be preferential selection for species to exhibit strong CAM or weak CAM, rather than intermediate metabolism.

P1848. Hydraulic vulnerability of Norway spruce trunkwood assessed by the hydraulic and the acoustic method

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Juvenile wood is not much appreciated by the wood industry because of its poor mechanical properties and its shrinkage behavior, but it may fulfill important physiological functions within the living tree stem. Two different methods to assess the hydraulic vulnerability of juvenile and mature Norway spruce (*Picea abies* (L.) Karst.) trunk wood were compared. Vulnerability curves were either based on the percent loss of conductivity versus pressure application using a pressure collar (hydraulic method) or on the cumulated acoustic emissions versus the relative loss of water (acoustic method). Acoustic emissions were detected in the high-frequency range of 100 kHz to 1 MHz during drying of sapwood samples at defined temperatures. Concerning the hydraulic method, juvenile wood showed lower hydraulic capacity but was less sensitive to dehydration than mature wood. Acoustic emission testing provided additional information about anatomical characteristics of the sapwood samples. The number of acoustic emissions detected in juvenile wood was much higher than in mature wood, indicating a higher number of tracheids per wood volume.

P1849. Synchronization and asynchronization of stem diameter changes during night transpiration in quercus crispula

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To evaluate water movement in tree stem, we setup eight opto-electronic dendrometers on the different parts of the stem from breast height to the peripheral region of crown of Quercus tree (6m height). We examined the changes of stem diameter during night time to avoid the effect of photosynthesis on water movement driven by the transpiration of leaves, and then described whether these diameter changes are synchronized or not among the eight measuring parts of stem.

In addition to the diurnal change of stem diameter (shrinkage in the day and swelling in the night), each stem showed short time diameter changes in the scale of 5 to 10 minutes, and the changes along time sequence are summarized with several types of synchronization levels among stems. Parallel directional changes, reciprocal directional changes, and independent directional changes were found depending of time periods, and these patterns indicate time dependent movement of internal water content of tree stems and its locality within tree parts.

P1850. Effects of low vigour-rootstocks on water relations and hydraulic architecture of seedlings of Olea europaea L.

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The research was aimed at studying water transport and the role of rootstock in the hydraulic architecture and water relations of young olive plants either self-rooted or obtained by grafting two cultivars i.e. Leccino "Dwarf" (LD) and Leccino Minerva (LM). The former, is characterized by low vigour and tolerance to low temperatures. Water relations parameters like diurnal time course of leaf conductance to water vapour (g_L), water potential (Y_L) and leaf osmotic potential at full turgor (Y_{po}), were measured during the summer period as well as the vulnerability to cavitation of 1-year-old stems and xylem conduit dimensions. Y_{po} measured in LD plant was about -2.5 ± 0.06 MPa in July and nearly the same in September. LM plants showed significantly less negative Y_{po} (Y_{po} = -2.07 ± 0.11 MPa). The water potential triggering stem cavitation (Y_{CAV}) was -1.7 MPa for LD plants but only -1.1 MPa in LM ones. Our conclusion is that dwarf olive clones (LD) seemed to be more resistant to drought than LM. These results are discussed in terms of water management of olive orchards and of mechanisms adopted by dwarf plants to resist seasonal water stress.

P1851. Xylem transport in stands of the common reed (Phragmites australis TRIN ex Steudel) growing in brackish water

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Water transport studies were carried out at Hiddensee (baltic sea) on a stand growing in brackish water. When intensively transpiring shoots were excised, stomata closed within 10 min. Xylem sap was collected using a hand-driven air pump. After excision the xylem sap rised from the sectioned internode vessels into the intact ones of the next internode within 1-2 min. Intact vessels above the sectioned internode remained sap-filled even after wilting of the leaves (osmotic pressure about 2 MPa), thus proving a strong cavitation barrier. Only a few culms of a dense stand were found to import acid fuchsin via the rhizome, after a culm was detopped under the dye solution and its xylem was connected with a reservoir of this solution. Some of the stained shoots were distant by more than 3 m from the dye infusion site. Obviously most shoots kept their water balance by own water uptake using adventive roots at the basal nodes. No dye entered the xylem at the root basis of the transfused culms, although the water potential of the brackish substrate was < -0.6 MPa. Results show, that sap flow can proceed against a strong osmotic force in the absence of a cohesion tension.

P1852. Cavitation events or artefacts? Ultrasonic signals emitted from conifer sapwood sections

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During dehydration, radial sapwood sections of *Juniperus virginiana* L. (Cupressaceae) produced ultrasonic acoustic emissions (UAE). Such signals are commonly considered to result from cavitation events in water-conducting elements. Varying settings of selected parameters, e.g. contact force between acoustic emission sensor and sample, gain of the monitor amplifier, and event-time-out, influenced the number of emissions detected.

With the total gain of the ultrasound counter set at 72 to 80 dB, an amplification range usually used in UAE measurements, and the contact force at 30 N, the numbers of intact tracheids present in a section (as calculated from section thickness, tracheid length, lumen diameter, cell wall thickness) were always higher than the numbers of emissions recorded. One possible reason is sound attenuation by living parenchyma cells. With gains over 80 dB, signal production increased continuously but results are no more considered reliable due to background noise.

Problems in acquisition and interpretation of UAE data are discussed and the acoustic technique is critically evaluated.

P1853. Xylem embolism and repair: what do we really know for sure?

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Xylem cavitation and consequent embolism is a common event in the plant's life and has been recorded in stems, roots and leaves of several species. Xylem embolism has been shown to be fully or partly reversed in plants of *Laurus nobilis* L. under low transpiration, even when pressures were still substantially negative. The loss of hydraulic conductance of stems was recovered by about 50% within 20 min after cavitation had been induced. The mechanism for such a rapid refilling is largely unknown. The present study reports measurements of sucrose, glucose and fructose concentration in the cortex, secondary xylem and sap as well as of the osmotic pressure of sap in precavitated stems of *L. nobilis* prior to and during xylem refilling. Our data show that all the three sugars increased in concentration, significantly only 2 min after embolism and increased further during the 15 more minutes required for the refilling to occur. Sugars were the product of starch depolymerization. The "osmotic" hypothesis for xylem refilling is, on this basis, confirmed in terms of active solute inflow into xylem conduits from vasiceentric cells, as possibly driven through a sugar/proton antiport.

P1854. Hydraulic redundancy in shrubs along aridity gradients in North and South America

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Arid regions around the globe are dominated by shrubs with strongly segmented woody roots and stems, which often physically split apart as the shrubs mature, resulting in complete axial segmentation of the plants' hydraulic system. We postulate that axial hydraulic segmentation is a form of hydraulic redundancy, which increases the chances for survival of the whole genetic individual under water-limited conditions, but at the cost of losing parts of the plants to drought-induced mortality. Based on this premise we hypothesized that the degree of hydraulic redundancy in shrubs will decrease along aridity gradients from arid to mesic conditions. To test this hypothesis, we documented the prevalence of morphological axis segmentation in shrubs from a variety of genera along North and South American aridity gradients located between 30° and 35° latitude. Along the same gradients we also measured axial hydraulic segmentation at the anatomical level by mapping pathways of water transport in the xylem using injected dye tracers. The results support our hypothesis that the degree of hydraulic redundancy in shrubs decreases from arid to mesic environments.

P1855. Are emboli any good? - The importance of gas in stems for oxygen diffusion and supply

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The presence of gas in xylem conduits is known to impede water transport, but could affect sapwood oxygen supply. In times of zero sapflow or flooded soils, when oxygen can not be transported upwards with the sapflow, the only source is the oxygen stored or diffusing radially through bark and xylem. We measured radial and axial diffusion of oxygen in wood of coniferous, ring-porous and diffuse-porous trees at different water and gas contents in the laboratory. The diffusion coefficient (D) in radial direction was between 10⁻¹¹ and 10⁻⁷ m²s⁻¹ and was significantly increasing with decreasing water content. Axial diffusion was one or two orders of magnitude higher. D was lowest in conifers, highest in diffuse-porous and intermediate in ring-porous hardwoods, where large vessels were mostly blocked by tyloses. Model calculations showed that at very high water content radial diffusion can be too low to assure an adequate supply of sapwood with oxygen. Also, due to the low solubility in water, ca. 150 times more oxygen can be stored in the same volume of air than in water. Thus a prime importance of gas in stems appears to be the supply of oxygen through storage and diffusion.

P1856. Rainfall absorption by the shoot of three Amazonian trees

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Water uptake by aerial organs is a potentially important mechanism of water acquisition by terrestrial plants in environments prone to soil water deficits. Here, we present patterns of sap flow in branches and trunks of 3 tree species in the Tapajós Forest, Amazônia (Brazil), that demonstrate direct foliar absorption of rainfall. We measured sap flow in branches and trunks of the 3 study species over a 2-year period using the heat ratio method, a sap flow technique that allows bi-directional measurement of water flow. During the first heavy rains of 2002, which occurred after a three-month dry period, reverse or acropetal flow in stems and branches of *Protium robustum* (breu), *Coussarea racemosa* (caferana) and *Manilkara huberi* (maçaranduba) were observed, a pattern that indicates absorption of water by the crown. The uptake of water by aerial parts may be advantageous for Amazonian species, because it may help reduce water stress at the sites of absorption, also supplementing soil water uptake, which in turn, can influence the performance, survival and persistence of these plants when exposed to extreme water deficits.

P1857. Refilling in Conifers at the Alpine Timberline

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Refilling processes enable the removal of embolism in plant water transport systems. We studied refilling in conifers growing at the alpine timberline, which are known to exhibit excessive embolism rates during winter.

Based on conductivity and water potential measurements as well as on dye perfusions, we analysed the dynamics of refilling in *Picea abies* trees at the timberline. Furthermore, we studied refilling of dehydrated young trees which were re-hydrated under various conditions.

At the alpine timberline, refilling was observed between late February and the end of May. During this period, loss of conductivity decreased from about 60% to 8% and water potentials increased from -2.4 to -0.2 MPa. In experiments, trees submersed in water showed some refilling within four weeks.

Results demonstrate that refilling does occur in conifers at the alpine timberline. As soil and stem base are frozen during the refilling period, an uptake of water by needles or over the bark seems likely. This hypothesis is also supported by our refilling experiments.

P1858. The preliminary study of drought stress on sorghum collection genebank of iran

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In order to study of low irrigation effect on iran sorghum collection gene bank, this germplasms with four check local planted in augment design in agriculture research station zahak - zabol . Total of germplasm derived from iran national plant genebank and number of germplasms were 128 . in this study all germplasm before panicle initiation , irrigation interrupted to harvest stage. Descriptive statistic results showed ,yield , stem diameter , panicle length and 100 seed weight had the most coefficient variation . Correlation coefficient between yield and stem diameter , panicle length and day number to flowering was significant and positive . Regression analysis by method backward indicated only stem diameter and panicle length remained in final model .Factor analysis showed three factors determined 72% from total variance among traits . Cluster analysis showed collection divided to four groups.

P1859. Effect of CaCl₂ on growth performance, photosynthetic efficiency and nitrogen assimilation of *Cichorium intybus* L. grown under NaCl stress

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Pot culture experiments were conducted to assess the extent of growth, photosynthetic efficiency and nitrogen assimilation of Kasni (*Cichorium intybus*) as affected by NaCl and CaCl₂ alone as well as in combination. Six treatments, i.e., 80 mM and 160 mM NaCl, 5 mM and 10 mM CaCl₂ and 80 mM + 10 mM and 160 mM + 10 mM of NaCl + CaCl₂ were given to the growing plants separately at three developmental stages, viz., the pre-flowering (30 DAS), flowering (120 DAS) and post-flowering (150 DAS) stages. Each NaCl treatment caused a significant reduction in total plant biomass, photosynthetic rate, stomatal conductance, total chlorophyll content, soluble protein content, NR activity and nitrogen content, although nitrate content increased. On the contrary CaCl₂ treatment gave a favorable effect, compared to the control. The effect of combined treatments was similar to that of NaCl but less in magnitude. The combined treatments thus mitigated the adverse effect caused by NaCl.

P1860. Influence of calcium on seed germination of *Arthrocnemum indicum* under chloride and sulphate salinities

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Arthrocnemum macrostachyum (Chenopodiaceae) is a stem succulent perennial halophyte commonly found in the subtropical salt marshes which are frequently inundated with seawater. Seeds were collected from a coastal tidal salt march located on the Arabian sea coast, Karachi, Pakistan.

Seeds were incubated in NaCl, Na₂SO₄, KCl, K₂SO₄, MgCl₂, and MgSO₄ (0, to 1000 mM) with various concentrations of Ca⁺⁺ (0, to 100 mM CaCO₃) at alternating temperature regime of 20-30 °C. Maximum germination occurred in distilled water. Low concentrations of different salts (200 mM) had little effect on germination and a further increase in salinity substantially inhibited germination. High concentrations of different salts (1000 mM) reduced seed germination substantially in *A. macrostachyum* however, application of Ca⁺⁺ alleviated the salinity effects. Rate of germination was low at high concentrations in the absence of Ca⁺⁺ however; the presence of Ca⁺⁺ increased the rate of germination under saline conditions. All Ca⁺⁺ concentrations alleviated the effect of salinity enforced dormancy in *A. macrostachyum*.

P1861. The effect of osmotic stress on lectin activity in bean (*Phaseolus vulgaris L.*) embryonic axis

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Many studies describe the accumulation of lectins under the influence of stress factors of various origins. The lectin activity dynamic during osmotic stress caused by draught were studied. Draught lead to increasing lectin activity in twice after 1 hour and in 11 times after 4 hours in isolated embryonic axis compared to normal conditions. Several lines of evidence implicate abscisic acid (ABA) in the control of lectin synthesis. The exogenous ABA influence on lectin activity was investigated as well. The effect of exogenous ABA (0,5 mg/l) during 3 hours before draught was similar to effect of osmotic stress leading to increase of lectin activity. In control exogenous ABA caused faster and briefer increase of activity in embryonic axis. At the same time combined action of exogenous ABA and draught caused weaker and more prolonged increase of lectin activity. Exogenous ABA in these conditions (before stress) can be the way of increase osmotic stress tolerance by accumulating defense proteins such as lectins.

P1862. The effect of Ca²⁺ on growth, accumulation of nutrient elements and electrophoretic patterns in *Descurainia sophia* salt stressed

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Calcium plays an important role in the resistance of plants to the salt stress. The effects of calcium salts at two concentrations on *Descurainia Sophia* which were under salt stress were studied. The chemical parameters was determined by using AA method. To assess the effect of salinity and Calcium treatments on electrophoretic polypeptide pattern, plant proteins were separated on SDS-PAGE gels. The results indicated that solutions containing 5 mM Ca²⁺ with 50mM NaCl have best effect on plant tolerance. Comparison between Polypeptide patterns showed that the Ca²⁺ effected proteins synthesis in plants. With the plants treated with 25 and 50mM NaCl, polypeptide bands (56 KDa) were more condensed than with the control. Bands of polypeptide with the MW of 66-170KDa were observed in plants treated with 25, 50 and 75 mM NaCl. These bands were not observed in plants pretreated with Ca²⁺.

P1863. The study of some physiological responses of *Atriplex canescens* to salinity

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The effect of salt stress on growth and some osmolytes production of xerohalophyte *Atriplex canescens* (Pursh.) Nutt in liquid culture solution was studied. Four weeks-old solutions grown *Atriplex canescens* plants were treated with NaCl of 0(control), 42.73, 85.47, 170.94, 256.41 and 341.88 mM in Humes nutrient solutions. The plants were grown under controlled environment. The plants were harvested after 5 weeks treatment for measurements of growth and biochemical analysis. Different growth parameters and the accumulation of different osmolytes in shoot and root were measured.

Growth not only did not decrease but also in some cases increased at salinity treatments. Treated plants tolerated at high concentrations of NaCl. At 170.94 mM NaCl different growth parameters were more than control. Root and stem proline contents increased with increasing of salinity but root protein

contents were lower than control. The contents of shoot protein increased with increasing of NaCl in medium and received to high concentration at 256.41mM NaCl. Root and shoot total soluble sugar contents significantly increased with increasing of salinity.

P1864. Influence of water availability and soil acidity on free proline levels in pigeon pea.

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Proline accumulation is not only associated to plants under water stress but also to the aluminium toxicity from soil acidity. Therefore, the physiological mechanisms involved in this response are possibly similar. The aim of this work to evaluate the influence of water availability and soil acidity on the levels of free proline in shoots of the pigeon pea cultivars 'IAPAR 43-Aratá' and 'IAC Fava Larga'. Four days after planting in sand with no water restriction plants were transferred to pots with the soil acidity treatments (pH in CaCl₂ = 5.9; 5.3; 4.8 and 4.1), and then submitted to 60%, 40% and 20% of water availability. The experiment was a 2x3x4 factorial with four replications arranged in completely randomized design, with cultivars, water availability and soil acidity as factors. Under severe water restriction the cultivar 'IAC Fava Larga' can be considered more tolerant because of its higher accumulation of free proline. The presence of aluminium associated to severe water restriction showed a synergistic effect on the levels of free proline. (Fapesp 01/03597-0)

P1865. Screening for drought resistance in Chickpea genotypes (*Cicer arietinum L.*)

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In order to evaluate drought resistance of chickpea genotypes, an experiment was conducted during 2003 in Mashhad and Nishabour regions. 34 genotypes were tested. Quantitative drought resistance indices such as Stress Tolerance Index (STI), Stress Susceptibility Index (SSI), Mean Productivity (MP), Harmonic Mean (HM), Tolerance Index (TOL) and Geometric Mean Productivity (GMP) base on yield in stress and non stress conditions were calculated. Results of regression analysis showed that MP, GMP, STI and HM indices have positive and high significance correlation with yield in stress and non stress conditions. These results indicate that MP, GMP, STI and HM are the most suitable criteria for screening drought resistance genotypes. Base on these criteria, genotype numbers of 4, 16 and 19 that have the Highest yield in stress and non stress condition and resting near to vectors of drought resistance indices like MP, GMP, STI and HM in multivariate biplot space, therefore these genotypes may recommended as drought resistance genotypes in this study.

P1866. PP2C type Phosphatase Regulates Stress-activated MAP Kinases

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Protein phosphorylation is mediated by action of protein kinases and protein phosphatases in eukaryotic signal transduction. Arabidopsis has 76 different PP2C genes. We isolated and characterized PP2C-type phosphatase from Arabidopsis and identified its substrate protein kinases. The data on MAPK (mitogen activated protein kinase) specific inactivation by cluster B member of PP2C family AP2C will be presented. We provide evidence that PP2Cs display exquisite substrate specificity for precise down-regulation of stress activated MAPKs. It demonstrates the mechanism for direct MAPK inactivation through dephosphorylation of specific phospho-threonine. Mutant analysis indicate that inactivation of MAPKs depends on the catalytic activity of AP2C. Comparative analysis was performed of this phosphatase and two other Arabidopsis PP2Cs, from cluster A, ABI2 and HAB1 in their ability to inactivate MAPKs. Yeast two-hybrid screen of cDNA library with AP2C and analysis of knock out and transgenic plants lines over expressing AP2C supports AP2C function on MAPK and propose a model of AP2C action in plant cells.

P1867. Allelopathic potential of *Sorghum bicolor* L. (Moench) genotypes against weeds

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abstract

Ten sorghum genotypes were screened for their allelopathic potential against weeds. The mechanisms by which it affects weeds, the persistence of the residues phytotoxins in soil and identification of phytotoxins in sorghum residues were conducted.

All sorghum genotypes significantly inhibited emergence and growth of *Lolium tennintum* seedlings. Giza, 15, Giza 115 and Enkath were found to be the highest allelopathic cultivars while cultivar Rabeh was the lowest. Residues of these cultivars when added to the soil at a rate of 3 and 6 g/kg soil significantly reduced seedlings emergence and growth of several weeds.

Field experiment showed that the allelopathic cultivars (Giza 15, Giza 115 and Enkath) exhibited a great ability to reduce weeds growth. The toxicity of sorghum residues persisted in soil for 10 weeks and reached its maximum at 4, 5 and 6 weeks of decomposition. Six phytotoxins (phenolic acids) were identified in the residues. The total phenolic acids were found to be higher in high allelopathic cultivars than in the low allelopathic cultivar (Rabeh).

P1868. Importance of Fertigation in new reclaimed lands in Egypt

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Fertigation (ferti-irrigation) is the frequent application of appropriate amount of designed fertilizers in irrigation water or through irrigation systems. Fertilizer is added in time when the crop needs it. Sprinkler and drip irrigation has increased during the last two decades in newly reclaimed areas. With increasing usage of these systems of irrigation a corresponding increase in fertigation has taken place. Such systems result in less water usage and better uniformity than in the cases of non-uniform furrow and flood systems.

Effective fertigation requires knowledge of certain soil characteristics such as pH, EC, soil texture, type of clay, plant characteristics e.g. optimum daily water usage, nutrient consumption rate and root distance in the soil. Irrigation water quality e.g. pH, mineral content, salinity and nutrient solubility is considered. Compared to the traditional method of fertilizer application, fertigation indicates more availability of nutrients to be absorbed by active root system, greater plant growth, high dry matter production, better crop uniformity and fruit quality, lower requirements of fertilizers.

P1869. Environmental gradients expressed in San Andrés Island mangroves, Colombian Caribbean

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We studied the relationship between the mangrove species in San Andrés Island, Colombia, and some environmental variables such as flooding level, salinity, pH, soil depth and texture. Those variables were measured through 74 plots of 500 m² each one, across 20 transects with length according to the extension of the six mangrove zones in the island. In order to establish species-environment relation we used Canonical Correspondence Analysis (CCA). We found a strong correlation between *Avicennia germinans* (L.)L., and soils with higher salinity values. *Conocarpus erecta* L. and *Cocos nucifera* L. were found in the soils with higher sand percentage, the species *Phyllanthus* sp., *Annona glabra* L., *Terminalia catappa* L. and *Ficus trigonata* L., were present in higher flooding levels environments, and a group of dry forests species was related to a coralline substrate in the mangrove of Hooker Bay. The island mangroves are dominated by *Rhizophora mangle* L., *A. germinans*, *Laguncularia racemosa* (L.)C.F.Gaertn and *C. erecta*, all of them widely distributed among Caribbean mangroves. Moreover other 17 species were registered associated to these ecosystems.

P1870. Cadmium Transport in *Salsola kali* tissues

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Salsola kali is a desert plant worldwide distributed. This plant is a potential Cd hyperaccumulator as it concentrated about 2000 ppm Cd in leaves. X-ray data showed that thiol related compounds are involved in Cd sequestration within the plant. In order to determine the mechanism involved, Cd content in phloem/xylem was evaluated and the response at the protein level. Plants were treated with 0, 20, 200, and 400 ppm Cd for 48 h in hydroponics media. The stem was used to separate the epidermis/phloem from the xylem/pith. Samples were lyophilized and Cd was evaluated in the two portions. Cd concentration was higher in phloem than that in xylem (7600 and 3000 ppm Cd, respectively) in 200 ppm Cd treatments. The protein profile in SDS-PAGE showed that in the presence of Cd, two peptides (of 29 and 14 kDa) were expressed. These protein bands were confirmed after G25 gel filtration protein and Cd codetermination. The nature of these peptides is currently being characterized.

P1871. The influence of water deficit on pigment and chlorophyll fluorescence of olive plants

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One-year-old rooted cutting Olive (*Olea europaea* L.) trees were grown in 14-liter pots containing a mixture of sandy loam soil, were subjected to various levels of water stress during two growing seasons in 2001 and 2002. Irrigation treatments were control (soil water at field capacity, $\psi_{soil} = -0.03$ MPa), medium stress ($\psi_{soil} = -0.5$ MPa) and severe stress ($\psi_{soil} = -1.5$ MPa). A factorial experiment based on randomized complete block design was used. Some characters like chlorophyll a, chlorophyll b, total chlorophyll and carotenoid and chlorophyll fluorescence were measured. Results showed that the degree of water stress had significant effect on the pigment degradation. The amount of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid showed a decrease under drought stress and this decrease under severe drought stress was significant in compare with other treatments. In present research initial chlorophyll fluorescence (Fo), maximum chlorophyll fluorescence (Fm), variable chlorophyll fluorescence (Fv) and chlorophyll fluorescence ratio (Fv/Fm) were increased, decreased, decreased and decreased respectively under drought stress.

P1872. The taxonomy and biology of mangrove swamp plant species in Nigeria

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Mangroves are a taxonomically-diverse group of plants which possess a common ability to survive and perpetuate themselves along sheltered tropical coastlines in saline environments under tidal influence.

The mangrove vegetation in Nigeria can be found throughout the south western region of the country, in contact with sea in places like Ikorodu, Forcados in Delta State, Akassa in River State, Opobo in Akwa Ibom and Calabar in Cross Rive State, interrupted by fresh water swamp forest in Niger Delta.

About twenty six (26) species in about fifteen families have been identified to thrive very well in Nigeria mangrove swamp, examples are *Rhizophora racemosa* (Rhizophoraceae), *Avicennia germinans* (Avicenniaceae) while associated species include *Conocarpus erectus* (Combretaceae), *Paspalum vaginatum* (Poaceae) etc.

The various species are able to survive in the harsh environment because they possess a wide range of morphological, anatomical and physiological adaptations which have elicited much scientific interest.

P1873. The Protective Function of Calcium, Cholesterol and Brassinosteroid on the Membrane of Rice Plant under Temperature Stresses

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In Southwestern China, rice (*Oryza sativa* L.) is often subjected to temperature stresses, the function of cell membrane and the ultrastructure of membrane of chloroplast and mitochondria were damaged gravely. A induced stress resistance of plant was observed when chilled rice seedling or high temperature and water stresses rice plant were treated with Ca, cholesterol and brassinosteroid (BR). It was found that electrolyte leakage and malondialdehyde contents decreased, the activities of superoxide dismutase, catalase and peroxidase increased and the structure of membrane of chloroplast and mitochondria were better. Chlorpromazine, a specific inhibitor of calmodulin (CaM), would inhibit Ca²⁺ function to protect cell membrane under temperature stresses. The result suggests that there is a relationship between Ca²⁺ function and CaM. Cholesterol and BR also raised heat stable protein and ATP contents, induced stress proteins. A wide range of influence of Ca, cholesterol and BR on rice physiological and biochemical characteristics, grain yield and yield components at the different rice growth stage was also studied under temperature stresses.

P1874. Environmental stress, altitudinal distribution and ecophysiology of Andean Plants from the Mediterranean Region of Central Chile.

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In Mediterranean ecosystems, species distribution and vegetation types are determined by several stress. We studied the combined effect of water, thermic and light factors on the photosynthesis, water balance and cold tolerance of evergreen trees (*Kageneckia oblonga*), semideciduous trees (*K. angustifolia*), shrubs (*Anartrophyllum cummingii*) and cushion plants (*A. gayanum*) in an gradient (1000-3100 m) in the Mediterranean Andes of Chile (33°). We hypothesized that the limiting factors would be dryness for trees, cold at the higher altitudes and the synergistic effect of light stress. For 3 years (2000-2002) we characterized the climate and soils and measure photosynthesis, transpiration, photoinhibition, pigments (xanthophylls), water potentials and tolerance or supercooling (temperature of damage by freezing) for the four species studied. The stress factors considered affect the plants differentially. We will discuss effects on plants distribution of conditions of climate changes.

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P1875. The production and characterization of salt tolerant substituted tritipyrum lines by genomic fluorescent *in situ* hybridisation (FISH)

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Soil salinity is a serious problem in the world. Wheat, although moderately is tolerant to salt, but can not be cultivated in many areas, however, some of the wild wheat relatives are highly tolerant, e.g. *Thinopyrum bessarabicum*. Eight hexaploid tritipyrum lines have been produced which can set seed in at least 250 mM NaCl. These tritipyrums (2n=6x=42, AABB^bE^b) due to a variety of reasons such as brittle rachis, late maturity, and meiotic instability will not fulfil the requirements of a successful commercial salt tolerant crop. To overcome these problems substituted tritipyrum, in which selected E^b chromosomes are replaced by D genome chromosomes of 6x wheat, was produced from 6x tritipyrum x 6x wheat hybrids (F1: 2n=6x=42, AABBDE^b). The fertile plants were screened by the genomic fluorescent *in situ* hybridisation technique to identify their E^b and D chromosome constitution. This study showed that the potential of producing tritipyrum with variable

numbers of E^b and D genome chromosomes is feasible and that FISH is a useful technique for determining the number of E^b chromosomes present.

Keywords: Tritipyrum, Fluorescent *In Situ* Hybridization, salinity

P1876. Functional responses to salinity of *Cochlearia* living along the coast of Brittany

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The genus *Cochlearia* grows in salt marshes and cliff, thus these plants must face to different levels of salt stress. This work reports the procedure for extraction, purification, chemical characterisation and quantification of organic solutes accumulated as stress response. In this study, twenty five populations from Brittany were investigated. These populations belonged to three well-known species, namely *C. aestuaria*, *C. anglica* and *C. officinalis*. Discriminant characteristics between these species were investigated through ecophysiological approaches. Organic solutes were extracted using methanol-water. When required extracts were partially purified, by treating with ion exchange resins or using a HPLC. The structures of these solutes were established by NMR spectroscopy. Solutes such as Proline, Glucose, Fructose, Sucrose and one glucoconjugate were identified. Solute levels were quantified by HPLC with ultra violet detection or refractive index. A possible correlation between these contents and the environmental conditions investigated.

P1877. Polyamines, development and stress response in plants : outcomes from the research on the subantarctic "Kerguelen cabbage"

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The subantarctic Brassicaceae *Pringlea antiscorbutica* or "Kerguelen cabbage" can tolerate adverse conditions from saline to freeze-thaw. However, *P. antiscorbutica* is sensitive to water stress under present climate warming and drying. We investigated growth regulators polyamines, which respond to abiotic stresses in plants. *P. antiscorbutica* displays high diversity for these metabolites. Amine metabolism in *P. antiscorbutica* showed a high responsiveness to temperature conditions, but some amines appeared sensitive to combinations of environmental factors. We showed that, in this plant, the response of polyamine metabolism to abiotic stress was effective in the field. Part of the polyamine pools could be allocated to the plant protection against adverse conditions. *N*-acetylputrescine, *N*-acetylspermidine and *N*-acetylspermine, which are characteristic of animals and bacteria, were detected at high levels in plants growing in low-altitude herbfields and this was possibly related to conditions of low water availability. Tyramine and dopamine were responsive to temperature conditions and a high tyramine level may be related to a slow plant growth.

P1878. High apoplastic solute concentrations in leaves alter water relations of the halophytic shrub, *Sarcobatus vermiculatus*

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It has long been known that many plants, particularly stress tolerant species, fail to equilibrate with soil water potential by dawn. While recent studies show that both nighttime shoot and root water loss can contribute to predawn disequilibrium (PDD), even when these are prevented PDD can be large. We predict that a major mechanism of PDD is high solute concentration in the leaf apoplast. We quantified leaf apoplastic solutes of the desert halophytic shrub, *Sarcobatus vermiculatus*, which has both large PDD and large discrepancies between predawn leaf and xylem water potentials. Pressure probe and nanoliter osmometer measurements confirmed leaf cell water relations consistent with psychrometric data. Analyses of apoplastic fluid collected by centrifugation showed 80±11 to 229±36 mM Na⁺, depending on salinity treatment, plus ~50 mM K⁺ in leaves at dawn. This demonstrates that leaf apoplastic solute concentration can be very

high, explaining discrepancies between leaf and xylem water potentials at dawn, as well as contributing to PDD. High apoplastic solute concentrations affect water relations and mineral nutrition of stress tolerant species with high PDD.

P1879. Selection of Rice (*Oryza sativa* L.) for Drought Tolerance under Field and *in Vitro* condition

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Seven rice cultivars, namely Khao Dok Mali 105, IR58821-23-B-1-2-1, CT9993-5-10-1-M, IR62266-42-6-2, IR57514-PMI-5-B-1-2, IR52561-UBN-1-1-2 and Jaw Hom Nin were screened under water stress condition in the field. These rice cultivars were divided into three groups. The first group which showed the highest tolerance to water stress was rice cv. CT9993-5-10-1-M. The second group which was moderately tolerance to water stress included rice cv. Khao Dok Mali 105. The last group which was most sensitive to water stress comprised rice cv. IR62266-42-6-2. In the second experiment, fresh weight, dry weight, percent survival, percent regeneration, osmotic potential, proline content, DNA bands and protein patterns of three rice cultivars namely Khao Dok Mali 105, CT9993-5-10-1-M and IR 62266-42-6-2 were investigated. These rice cultivars were cultured on MS medium supplemented with various concentrations of polyethylene glycol or mannitol. It was found that fresh weight, dry weight, % survival, % regeneration, osmotic potential in all three rice cultivars decreased whereas proline content increased.

P1880. Productivity and water relations of corn plants grown under different conditions of water supply and potash fertilization

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Two field experiments were conducted in Shalakan, Egypt, during 2000 and 2001 seasons, to study the effect of four irrigation treatments, i.e. 80, 60, 40 and 20% of available soil moisture (ASM) and four potassium levels, viz. 57,86,115,144 kg K₂O/ha on corn. A split plot design with four replicates was used, where irrigation levels were run at the main plots and potassium in the sub ones. The study included 16 traits in addition to some water relationships. The obtained data showed that irrigation gradually increased products of most traits, including grain yield. No. of rows/ear did not respond to irrigation varying, meanwhile shelling % increased as irrigation level was reduced. Potassium addition up to 86 kg/ha reflected in positive effect on most studied aspects, including grain yield. It was concluded that 80% ASM in combination with 57 or 86 kg K₂O/ha produced the highest grain yields. Water consumptive use (WCU) increased by increasing irrigation and through hot seasons. The value of (WCU) was ranged between 3663 to 6123 m³/ha. The treatments 80% ASM, 57 kg K₂O/ha and their combination produced the highest values of water use efficiency (WUE) in both seasons.

P1881. Transcripts expression of two groundnut (*Arachis hypogaea* L.) varieties tolerant and sensitive to drought

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Groundnut (*Arachis hypogaea* L.) is an important crop in the semi-arid Tropics. It is often exposed to extremely severe climatic conditions. Little is known about its molecular responses to drought although water deficit-induced genes have been described in other plant species.

Using RT-PCR on a system including a drought-sensitive and a drought-tolerant variety, expression of genes involved in protein turnover, phospholipid degradation and membrane protection was studied under water limiting conditions. Results showed that genes encoding a cysteine protease and phospholipase D were over-expressed while a serine protease gene was repressed and that of a LEA protein induced by drought. For all but the LEA gene, expression patterns in response to progressive drought-stress differed between the tolerant and sensitive variety. Therefore,

studying genes with known functions allowed us to identify genes controlled by drought in groundnut and to better understand how this species copes with drought-stress.

P1882. Multicystatins are induced by drought-stress in Cowpea (*Vigna unguiculata* (L.) Walp) leaves

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Phytocystatins are proteinaceous inhibitors of cysteine proteinases (CP). CP can be induced by abiotic stresses resulting in cell protein autophagy. To study involvement of phytocystatins in plant response to abiotic stresses, we isolated a full-length cDNA encoding a two-domain cystatin from cowpea (*Vigna unguiculata* (L.) Walp.) leaves and studied cystatin expression pattern in response to drought-stress in two cowpea cultivars with different capacity to resist drought (EPACE-1, resistant and IT83, sensitive). Our results showed that both cultivars developed an inhibition-based control strategy towards cysteine proteinase activities, involving at least two cystatin messengers and proteins as revealed by Northern and Western analyses, respectively. Furthermore, in the resistant cultivar, this inhibitory strategy appeared to be carried out further, in terms of water deficit intensity. This could minimize protein loss and allow leaf cells to adapt to drier environments and/or to recover more easily at the end of the drought.

P1883. Diluted Seawater and Benzyl Adenine Effects on Macro and Micro Nutrients States in Barley Shoots

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A pot experiment was conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt during 2000/2001 winter season to study the effect of Benzyl adenine (spraying 50 ppm and without) and salt stress [tap water (250 ppm), 2000, 4000, 6000 and 8000 ppm from mixed diluted seawater by fresh water] on macro and micro nutrients concentration and contents in shoots of barley plants. All micro-nutrients contents i.e. N, P, K, Ca and Na lowered by salinity. Mn and Zn values showed approximately the same response. Fe content was increased by salt stress up to 2000 ppm and tended to decrease by increasing salt concentration up to the highest level used. Spraying BA in the rate of 50 ppm led to significant decreases in N and Ca content but P and K contents were reversely responded. Zn content was lowered, however, Fe and Mn contents seemed to be without effect by BA treatment compare to the control. The interaction effects of BA and salt stress in the nutrient concentration and contents were discussed.

P1884. Polyamine Metabolism in Response to Drought and Cold stresses

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The oldest known and perhaps the most dramatic changes in polyamine (PA) metabolism are those obtained when plants are challenged with different abiotic stresses. For many years, the nature and physiological meaning of the massive PA accumulation under these challenging conditions have remained ill-defined. The relevance of such metabolic pathway in the plant response to abiotic stress has just been recently approached with the use of *Arabidopsis* as a model plant, since it allows global approaches to study the function of PA metabolism. In our lab we started a multidisciplinary approach based in transcriptomics, metabolomics and functional genomics in order to gain insight into the role of PA metabolism in plant abiotic stress responses. Gene expression analyses by real-time RT-PCR of *Arabidopsis thaliana* plants subjected to cold and dehydration stresses have identified the key PA metabolic genes which expression is differentially regulated by these environmental insults. Metabolomic data have also identified specific PA profile for each type of stress. The function of key genes in such stress responses has been analyzed by using gene-disruption mutants

P1885. Photosynthetic activity of *Vaccinium vitis-idaea* during winter

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The photosynthetic activity of lingonberry (*Vaccinium vitis-idaea*), a common evergreen dwarf shrub in boreal forests, was studied during winter. The leaves of lingonberry maintained substantial photosynthetic capacity throughout winter, the lowest light-saturated rates of gross photosynthesis measured being approximately $1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. High values of the Fv/Fm ratio of chlorophyll fluorescence induction (ca 0.73) and stable low electrolyte leakage percentage of the leaves suggested that shoots covered by snow did not suffer from winter stress. Immediately after snow melt, however, Fv/Fm ratios decreased to approximately 0.3. The decrease was accompanied by a clear reduction in CO₂ uptake, probably due to photoinhibition of leaves exposed to high light at relatively low temperatures. The recovery of both photosynthesis and chlorophyll fluorescence required several weeks. The results suggest that a protecting snow cover is a key factor affecting the wintertime photosynthetic activity of *Vaccinium*.

P1886. Ecophysiological Strategies of Desiccation-Tolerant Crustacean Cryptogamic layer on Tropical Inselbergs

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The bare calcareous and quartzitic rock surfaces of inselbergs are generally pioneering inhabited by a complex community of cyanobacteria, algae, fungi and lichens. Although these cryptogamic plants constitute a prominent part of the biofilm of exposed rocks in the tropics and subtropics, and show a luxuriant growth in the extremely conditions, their diversity and ecophysiological strategies to withstand long periods of drought in a dehydrated state are poorly investigated. The degrees of water availability in different habitats establish the different growth forms of cryptogamic plants, and the growth forms of the biofilms influence the photosynthetic behavior including the carbon gain under different water regimes, and finally determine the different distribution patterns and the color of inselbergs. The time-scale ecophysiological responses, especially the photosynthetic changes of the biofilm on the rocks to the rehydration are still need further studied.

P1887. Proteolytic activities in response to water deficit in desiccation-tolerant and desiccation non-tolerant species of the family Gesneriaceae

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In the response of plant to drought, the control of protein breakdown and cellular proteolytic activity is held to be very important. We have studied protease activities in the desiccation tolerant flowering plant *Ramonda serbica* Panc. Severe water deficit led to desiccation (3% relative water content) and induced a marked increase of aminopeptidase and serine protease activities in *Ramonda* leaves. These increases were fully reversed within 24 hours on rehydration. The increase of both endo- and exopeptidase activities strongly suggests a requirement to remove proteins that accumulate on water removal and to provide a source of recycled amino acids for resynthesis of proteins on rehydration. *Saintpaulia ionantha* is a member of the same family but it is not tolerant to desiccation. In watered plants it exhibits a similar profile of protease activities to that measured in *Ramonda*. A comparison of the influence of the first stages of dehydration will be reported.

P1888. Changes in spectral reflectance of some foliar lichens during desiccation

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Remote detection of water status and photosynthetic or photoprotective pigments using analysis of spectral reflectance in visible and near infrared spectral regions would be very helpful in

ecophysiological study of lichens. Specific surface morphology, anatomy and deposition of secondary metabolites in lichen thalli may complicate applicability of reflectometric approaches well elaborated for leaves of seed plants. We have investigated validity of several reflectance indices for detection of water status in some foliar lichens (*Umbilicaria hirsuta*, *U. antarctica*, *Lasallia pustulata*). In spite of extremely high increase in total reflectance during desiccation, caused mainly by structural changes in the upper cortex, it was possible to derive useful information by a narrow-band analysis. The depression in reflectance around 970 nm and its derivative characteristics were the most reliable predictors of water status. They were in close correlation with both the water content and the water potential of the thalli within a broad range of hydration from full saturation to critical water shortage at which photosynthetic processes were inactivated.

P1889. Leaf structure and soluble carbohydrate composition of pteridophytes from rock outcrops of Rio Janeiro State, Brazil.

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Rock outcrops are prominent sites for desiccation tolerance research. Studies concerning this topic in pteridophytes have been focusing mainly northern *Selaginella* species, and little is known about desiccation tolerance processes in other pteridophyte groups. *Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) Mickel and *Anemia villosa* Humb. et Bonpl. ex Willd. are desiccation tolerant ferns commonly associated to vegetation islands on rock outcrops located in Rio de Janeiro. In this work, leaf anatomy and sugar composition of these species was analyzed. Despite the environmental restrictions imposed in rock outcrops, both species have leaf blades with mesomorphic characteristics, that could be important on structural integrity maintenance during dehydration curling, contrasting to xeromorphic petiole, which cortical cells store protective substances. Glucose, fructose and sucrose, are the main sugars present in whole leaf water extracts of both species. The results show how structure and biochemical features may be contributing for the establishment of these species in habitats with water shortage.

P1890. Ecophysiological investigation of tropical desiccation-tolerant vascular plants

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The main goals of the project were to describe the main ecophysiological processes of some poikilohydric desiccation-tolerant (DT) vascular inselberg plants. It is extremely important to examine DT plants on the whole globe to gain better knowledge on the drought tolerance. Morphological and ecophysiological processes of selected tropical flowering plant species were carried out on widely distributed tropical poikilohydric species, like *Afrotrilepis pilosa*, *Coleochloa microcephala* and *Xerophyta scabrida* under *in situ* natural habitat like greenhouse conditions. The selected taxa are suitable objects for ecophysiological screening, as well as representative for characteristic tropical region. Desiccation and revival afterwards very drastically influence the whole photosynthetic system of these plants, hence the present study mainly focused on the photosynthetic processes. The *in situ*-like greenhouse examinations included gas exchange and fluorescence measurements during desiccation and revival processes as well as metabolically active stage.

P1891. Ecophysiological differences between the desiccation-tolerant species *Ramonda serbica* and *R. nathaliae*

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The presence in the Balkan peninsula of several poikilohydric flowering plants belonging to the *Gesneriaceae* family provides the possibility to investigate comparatively the unique phenomenon of desiccation tolerance and to establish the specificity, degree and

pattern of their resistance. This is also important for protecting these Balkan endemo-relict plants.

We studied the species *Ramonda serbica* and *R. nathaliae*, pointing out their chorological differentiation in the Balkans, ecoanatomical differences, electrolyte leakage of leaves subjected to dehydration. The pattern of chlorophyll metabolism of these homoiochlorophyllous species during dehydration and rehydration was also studied. Special attention has been paid to *R. serbica*, with respect to its photosynthetic activity, defence systems against oxidative stress, plasma membrane lipids and low-molecular weight substances during dehydration and rehydration. Similar investigations on *R. nathaliae* are in progress.

P1892. Photosynthetic features of desiccation tolerant moss species native of CO₂ spring

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So far many long and short term high-CO₂ experiments were run in growth cabinets however none of these studies could answer the basic question of how plants would respond to chronic atmospheric CO₂ enrichment. The natural CO₂ springs permit studying the real long-term effects on plants or plant communities. Since long-term effect on adaptation of photosynthetic system of poikilohydric species are less known our aim was to investigate desiccation tolerant forest-floor moss species growing around a CO₂ vent as well as a nearby control site in the vicinity of Gornja Radgona in Slovenia. The study was carried out on five indigenous species. The CO₂ assimilation showed upward acclimation in every CO₂ spring samples. There was no noticeable difference in Fv/Fm values while RfD was higher in four vent samples. The photosynthetic pigment content differed considerable only in two species, but it increased at the majority of the species at the vent. The carbon content and C/N ratio were also higher in the high-CO₂ moss samples. The results suggest that long-term adjustment of photosynthetic features to elevated atmospheric CO₂ may be diverse and definitely species dependent.

P1893. Desiccation Survival Times in Different Desiccation-Tolerant Plants

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There is no exact information about the longevity of the desiccated period after what the desiccation tolerant plants can recover their photosynthetic structure and function. Generally, longer the period spent in a desiccated state lesser the potential to full metabolic recovery. We know just vascular DT-s can survive longer desiccated periods than cryptogams. Therefore, in the case of three cryptogam DT species: the moss *Tortula ruralis*, and the lichens *Cladonia convoluta*, and *C. furcata*, and two vascular monocotyledonous DT species: *Xerophyta scabrada*, and *Coleochloa microcephala* were examined for the recovery of their net CO₂ assimilation during rehydration (following stored in dry stage for different lengths). According to our results the cryptogams were able to recover their photosynthetic activity just after 4 months or less, while the leaves of *X. scabrada* and *C. microcephala* remained able to revive after 2-11 years of desiccation.

P1894. The role of growth regulators in formation of plants adaptive potential to unfavorable environmental conditions

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The mechanisms of plant adaptive reactions induced by such adverse factors as high (+42°C) and low (+8°C) positive temperatures and ions of heavy metals (Cd²⁺, Pb²⁺) under influence of Ukrainian growth regulators were investigated. The action of extreme factors resulted in changes of endogenous phytohormone balance, lipid peroxidation process (LP) intensity and antioxidation enzyme systems activity (peroxidases, catalases). Inducing of the protein polymorphism under different stress conditions was established. Growth regulators (ivin, emistym C and agrostimulin) influence on physiological and biochemical functions and promote plant adaptation on stress.

P1895. The role of growth regulators in formation of plants adaptive potential to unfavorable environmental conditions

O. I. Terek, O. Velychko, N. Yavorska;
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P1896. Reconsidering leaf structure vs. physiological traits relationships: a comparison between species with heterobaric or homobaric leaves, growing in Mediterranean environment

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Heterobaric leaves (HEL), contrary to homobaric (HOL), are characterized by bundle sheath extensions forming a transparent area network on their lamina. Although photosynthetically inactive, this network enhances photosynthesis by transferring light in deep internal mesophyll layers.

Here, the leaf structural strategies of more than 40 species belonging to either HEL or HOL groups were compared in respect to physiological traits. HEL, contrary to HOL, showed a positive correlation between LMA and thickness. Furthermore, HEL had significantly higher N concentration, as well as greater investment of nitrogen per leaf area with increasing LMA, than HOL. Moreover, both the negative PNUJ to thickness and the positive thickness to transparent-area volume correlations indicate a high structural N-cost of the latter. Still, the photosynthetic capacity of HEL, expressed per unit (projected) area, increased and did not differ significantly from that of HOL. Given the significantly ¹³C-enriched HEL, compared to HOL, corresponding to higher WUE, it is implied that although N-demanding, transparent areas formation is a response for improving leaf water status.

P1897. The influence of the photoperiod on redox regulation and stress responses

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Under natural conditions plants are subjected to a changing environment. Plants possess a large set of mechanisms, e. g. the malate valve, to prevent damages in the short term while persisting alterations in the redox state induce a long-term response on the transcriptional level. To investigate the different acclimation responses, Arabidopsis plants grown in low light either in short days (SD) or long days (LD) were transferred into high light at 12°C. In SD plants, the NADP-MDH activation state increased, and its protein level was up-regulated a few hours after the onset of stress. Array analysis showed an increase of glutathione metabolism and some photosynthesis-related genes. The cellular glutathione content increased at a constant redox state. In LD plants, only the expression of several antioxidative enzymes increased strongly. We conclude that the endogenous systems that measure the day length interact with redox regulation, and override the interpretation of the signals, i. e. they redirect redox-mediated acclimation signals from a more efficient light usage and from redox poisoning in SD towards the avoidance of oxidative damage under LD conditions.

P1898. Isolation and characterization of yellow stripe1-like gene family in rice

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Plants release ferric chelating phytosiderophores and then transport ferric-phytosiderophores complexes across the plasma membrane by the yellow stripe1 (YS1). We report the isolation and

characterization of the rice YSL (*yellow stripe1-like*, designated OsYSL) genes. There are 18 members in rice. Their expression patterns under the micronutrient deficiency were analyzed by RT-PCR. From the pool of rice T-DNA insertional lines transformed with pGA2715, we have identified an activation tagged mutant of OsYSL16. To analyze the phenotype of OsYSL16 activation line, we tested the iron deficiency tolerance by germinating and growing the plants on a solid medium without iron. Visual differences were reflected by the chlorophyll contents, indicating that the OsYSL16 activated line is more tolerant to iron deficiency than wild type. Iron content of the OsYSL16 activated line is similar to that of wild type. RT-PCR analysis indicated that OsYSL16 gene is preferentially expressed in leaves. Our results suggested that the function of OsYSL16 in iron homeostasis is more related to the distribution of iron through the tissues than the uptake from the soils.

P1899. PS 2 thermotolerance in relation to ice nucleation, frost and heat damage in evergreen subalpine plants during winter

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PS 2 thermotolerance was compared to ice formation, frost and heat damage of evergreen subalpine plants (*Picea abies*, *Pinus cembra*, *Rhododendron ferrugineum*, *Saxifraga paniculata*) under naturally low (winter) and high (summer) PS 2 efficiencies.

Basic chlorophyll fluorescence (F_0) was recorded during freezing (+2°C - -10°C at 2°C/h). On extracellular ice formation (-4 to -7°C) a significant, sudden increase in F_0 was observed. This was not caused by frost damage that occurred below <-21°C at least in winter. Also surface ice had no effect on F_0 . F_0 measurements are hence a useful tool to distinguish between surface ice and extracellular ice inside the leaf tissue which cannot be differentiated by freezing exotherms.

PS 2 heat tolerance was determined by T- F_0 curves (20-65°C at 1°C/min). With $F_V/F_M > 0.8$ two F_0 maxima were recorded. The first corresponded with tissue heat damage (46.9-54.3°C), the second occurred above 60°C. With $F_V/F_M < 0.3$ only the second F_0 maximum was detectable leading to an overestimation of heat tolerance by >10°C. Under conditions with low F_V/F_M PS 2 heat tolerance cannot be determined via T- F_0 curves.

P1900. The effect of gamma irradiation on cytological and physiological function of two cultivar of barley

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An investigation was performed in cytogenetic lab. of Nuclear Agriculture- Atomic Energy Organization of Iran in 2004-5. In this research the effect of gamma irradiation on cytological and physiological function of two cultivars of barley were examined. For this aim cytological and physiological sensitivity of two cultivars (30109, 30130) were assessed by different gamma radiation doses (50,150,250,350,450 Gy). By cytological studies in addition to defining the karyotype, the rate of chromosomal aberrations due to the effect of irradiation was studied and it was observed that the chromosomal aberrations increased by increasing the rate of irradiation. In 350 and 450 Gy were observed more different forms of chromosomal damage such as ring & dicentric chromosome, deletion and Translocation than the other dose. The results showed that by increasing gamma ray dose, the growth rate, root and shoot length of two cultivars were decreased, and germination percentage had no significant difference. This work suggested that the increasing of chromosomal aberrations, so decrease mean value of growth rate.

P1901. ABA signal transduction in guard cells: systems biology and key players.

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Guard cells regulate stomatal apertures by responding dynamically to many environmental and endogenous signals, including the stress hormone abscisic acid (ABA). ABA-induced stomatal closure is one of the best characterized signal transduction processes in plants, but a predictive model is still not available for this complex multistep process. We have synthesized over 140

experimental observations into a consistent guard cell signaling network for ABA-induced stomatal closure, and have modeled this process using Boolean methods. Our dynamic model captures several essential features of this biological system, such as the existence of complementary signaling pathways. We are combining genetic, biochemical and cell biological assays to probe one key aspect of this signaling network, consisting of the signal transducing heterotrimeric G protein and its interaction partners (Curr Opin Plant Biol. 7: 719-731). We have found that plants harboring T-DNA mutations in genes encoding proteins of the G protein complex exhibit altered stomatal sensitivity to ABA. Supported by NSF MCB-0209694 & MCB-03-45251 (S.M.A.), and by a Sloan Research Fellowship (R.A.).

P1902. Gene Expression Patterns of Stress-induced PP2C Phosphatases

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Cells are communicating by signals generated at cell membranes, transmitting/processing the information and responding selectively to diverse inputs. Strategy exercised in these transmissions is based on reversible protein phosphorylation that is mediated by concerted action of protein kinases and protein phosphatases. The phosphoprotein phosphatases of PP2C-type are involved in signalling pathways, including abiotic stress conditions. They were found to regulate stress-activated MAPKs in plants by displaying exquisite substrate specificity. We isolated, characterised and addressed the developmental regulation of two Arabidopsis PP2C phosphatases by promoter-reporter gene approach. Promoter activity associated to stomata suggests that PP2C may regulate MAPK pathways in these cells.

To characterize further these phosphatases we produced transgenic plants overexpressing epitope tagged proteins and analysed T-DNA insertion lines.

P1903. Cadmium Toxicity Is Associated with Oxidative Metabolism Dysfunction and the Production of Oxyradicals in Zea mays L. Seedlings

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The aim of the study is to test the hypothesis that Cd²⁺ induces maize defense pathways against abiotic stresses. Different physiological and developmental changes were studied in roots of hydroponically grown maize seedlings. Antioxidant enzymes were studied to know the possible involvement of Cd²⁺ in oxidative stress. Cd²⁺ produced a significant inhibition of growth as well as reduction in pigments. An alteration in the activated oxygen metabolism were detected and evidenced by reduction in sulfhydryl group as well as a decrease in total glutathione. Moreover, the accumulation of H₂O₂ due to imbalance of redox and inhibition of antioxidant system. CAT, APx and POX were enhanced up to 50 µM Cd²⁺ supply. Roots challenged by Cd²⁺ at concentrations exceeding the detoxification capacity exhibit an increase in the rate of cell death and accumulation of soluble phenolics, and a decrease in dry weights as well as leaf area. Cd²⁺ induced oxidative stress indicated by the accumulation of MDA result from lipid peroxidation and significant increase in the SOD activity. Cd²⁺ did not cause injury in root but expedite differentiation, accelerating aging.

P1904. Effect of Phosphorus on soluble sugars, Phenolic compounds and Lipids of Brassica napus L.

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Brassica napus knows a very important oil seed plant specially for its edible oil. An attempt was made to study the effects of P on sugar, phenolic compounds and lipids of Brassica napus seeds were germinated in the soil in pot.

Treatments of employed when seedlings were three leafleted. Different concentrations of P in the form of NaH₂PO₄ as 0, 0.02, 0.2 and 2M were added to the soil once.

After 5 weeks leaves and 8 weeks seeds samples were collected from plants. Soluble sugar, phenolic compounds of leaves and lipids of leaves and seeds were measured.

The data analyzed with MSTATC program. DMRT showed that soluble, sugar, phenolic compounds, unsaturated/saturated ratio of fatty acids and also total lipids of leaves and seeds significantly (except sugar) increased by addition of P. The amount of fatty acids of seeds of plants grown in 0.02 M P significant ($p < 0.05$) increased.

It can be discussed that increasing of sugars and phenolic compounds which synthesized in photosynthesis are involved P synthesis of lipids is also related to sugars and phenolic compounds protect lipids against oxidation.

Generally addition of P in brassica napus increased secondary metabolite.

P1905. Hepatoprotective effect and antioxidant role of Methanol extract of *Mucuna pruriens* seeds on Paracetamol intoxicated rats

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The methanol extract of the seeds of *Mucuna pruriens* (Family: Fabaceae) was evaluated for hepatoprotective activity against paracetamol induced liver toxicity in rats. The levels of serum glutamate oxaloacetate transaminase (SGOT), pyruvate transaminase (SGPT), alkaline phosphate (SALP), bilirubin, total protein, creatinine and blood urea were determined. The paracetamol induction at the dose of 500mg/kg/day resulted a significant elevation in the levels of serum enzymes, bilirubin, total protein with decreased blood urea. An intraperitoneal administration of methanol extract of *Mucuna pruriens* (MEMP) seeds at the dose of 125mg and 250mg/kg body weight for 9 days exhibited significant ($P < 0.01$) protective effect by decreasing SGOT, SGPT, SALP, bilirubin, total protein, and creatinine. The results were found to be in a dose dependent manner. Further, the study was extended to estimate the effect of MEMP on the levels of lipid peroxidation (LPO), reduced glutathione (GSH), catalase (CAT) and total protein *in vivo*. The MEMP significantly ($P < 0.05$) decreased the levels of thiobarbituric acid reactive substances (TBARS) and increased levels of reduced glutathione (GSH), and the activities of Catalase (CAT) and total protein resulted in reducing the free radical formation in liver. The effects of MEMP were compared with a standard drug Silymarin at the dose of 25mg/kg. body weight.

P1906. Study on the germination of BELLA-DONNA L. seeds in West Mazandaran, Iran.

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From ancient times *Atropa bella-donna* L. is well known medicinal plant with roots, leaves and stem used as herbal drugs. The whole plant contains tropan alkaloids mainly atropine and a small hyoscyamine, scopolamine and apatropine. A new row material has been collected from habitats for the need of pharmaceneitic industry. This plant is one of the most important medicinal plant which are growing wild in west of Iran in Gorgan, Ramsar, tonekabon, Gilevan, Roudbar, Tavelesh and Asalem. In this study sampels were collected from Kandovan.

The seed germination was studied with effect of scarification, variable temperature and treatments Giberlic Acid (GA₃). The present study showed effect of scarification is insignificant but variable temperature (6 hour at 30 °c and 18 hour at 15 °c) simulated significantly seed germination (82.5%) and with Giberlic Acid (GA₃) 1mg/l H₂O (89.5%).

P1907. Effects of different rates of nitrogen on yield, protein and nitrate accumulation in forage of two varieties CORN (*Zea mays* L.) hybrids.

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The objective of this study was to examine plant morphological characters and the forage yield, protein and nitrate accumulation on responses of two corn hybrids under effects of four different rates of nitrogen fertilizer. Two single-cross hybrids maize varieties, KSC 604 (mid-season) and KSC 704 (late-season) were planted at plant density (70000 plant/ha) and four nitrogen fertilizer rates (0, 300, 450 and 600/ha) as urea (0, 175, 225 and 300 kg N/ha). A split plot design was used with four replications which, hybrids as main plot and nitrogen levels as sub plot. In this experiment forage yield and its components and some attributed characters such as total number of leaves, number of ear, plant height, number of tassel, leaf as

compared with stem, total dry matter, harvest index, nitrate accumulation in plant, soil and protein were evaluated. The analysis of variance shows that main effects and their interactions were observed significantly. Some of main results are: with increasing nitrogen fertilizer, forage yield and nitrate accumulation increased. highest forage yield with lowest nitrate in both varieties were obtained at 450 kg urea/ha.

P1908. Influence of environmental factors on seed germination of *Salvia sclarea* under laboratory conditions (collected from Roudbar, IRAN).

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Evaluation of temperature, resistance to salinity and water stress was study on germination of *Salvia sclarea* seeds under laboratory conditions. seed sample was gathered from Roudbar ecotype. Start of germination was in 0 °c. max percentage was in 4 and 8 °c. from 12 to 24 °c percentage of germination was reduced. Rate of germination increased from 0 to 12 °c and in the latter temperature, it was maximum and in temperature higher than 12 °c. rate of germination was reduced. Percentage of germination was maximum in control and with increasing of osmotic potential (NaCl), it was reduced. In this case, there was not statistically significant differences from -6 to -3 bar from -12 to -9 bar, with increasing osmotic potential, rate of germination was reduced. In this case, control treatment had significant difference with other treatments but there was not significant difference between other salinity levels but the trend was decreasing.

P1909. Interacting effects of climate change on sagebrush (*Artemisia tridentata*) seedlings

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Seedling response to climate change has often been ignored. Sagebrush (*Artemisia tridentata*) is common in the Western United States, where variability in climate is expected to increase. I grew two subspecies of sagebrush, one native to mountains (Mountain sagebrush) and the other to the desert (Basin sagebrush). I studied growth and photosynthesis of seedlings exposed to two levels of soil moisture in combination with freezing. Under high moisture, photosynthesis of Mountain sagebrush was twice that of Basin sagebrush. Drought reduced photosynthesis of Basin sagebrush by 75% and Mountain sagebrush by 60%. In contrast, freezing reduced photosynthesis of Mountain sagebrush by 78% while Basin sagebrush was unaffected. However, droughted Basin sagebrush was more sensitive to freezing, which reduced photosynthesis by 50%. In contrast, drought did not affect the susceptibility of Mountain sagebrush to freezing. Subspecies native to contrasting environments may have dissimilar responses to various aspects of climate change. Furthermore, the susceptibility of plants to an aspect of climate change may be modified by other simultaneously occurring factors.

P1910. Dependence of soil microorganisms on recent photosynthates from trees

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We studied the dependence of the carbon status of soil microorganisms on the supply of current photosynthates from the tree canopy in a boreal forest. We used the method SIR (Substrate Induced Respiration) in a girdling experiment, using sucrose from a C₄ plant which had a c. 16‰ more positive ¹³C value than the boreal "C₃-soil". The respiration rate after sucrose application was on average c. 1.7 times higher in both girdled and non-girdled plots than the basal respiration during the course of three days. In non-girdled plots, respiration of added C₄-sucrose accounted for 25% only of total respiration after sucrose application, whereas it accounted for 50% in girdled plots. The increased release of C₃-carbon after amendment with C₄-sucrose is viewed as increased respiration of C already present in the microbial biomass. Our data thus show that less C is available to the microorganisms in girdled plots and heightens the importance of recent plant photosynthates for soil processes.

P1911. An investigation of the allelopathic effects of *Cressa cretica* on the germination and growth of purslane, goosefoot and oat seedlings

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Cressa cretica belongs to *Convolvulaceae*. This plant is annual herb, which grows on salty soils. Plant collected from Yazd province in Iran. For this the root and shoot of the plant dried and powdered separately. The extract of 2%, 5% and 10% was prepared from the root and shoot powder. Then 3 kinds of seed of weeds including *Portulaca oleracea*, *Chenopodium album* and *Avena sativa* planted in the prepared extracts in petridish on watman paper. This project is done in a completely randomized design with 3 replication (for every replication 20 seeds). Also 20 seeds from every weed planted in distilled water as a control group. After 72 hours the number of germination seeds counted and after a week seedling high measured.

According to the results and statistical analysis, *Cressa cretica* is an allelopathic plant. 10% extract of shoot showed the strongest allelopathic effect and 2% extract of root showed the weakest. In the following the strongest to the weakest allelopathic parts of *Cressa cretica* in related to seeds germination is shown: 10%shoot>5%shoot>10%root>5%root>2%shoot>2%root>distilled water

P1912. Effect of Substituents of Some Synthetic Monoethanolamine Salts of *para*-Substituted Benzoic Acids (MEASPBA) during Cucumber (*Cucumis sativus* L.) GerminationM. E. Crisan¹, C. M. Berteau², M. Grozav¹, L. Kurunczi¹, M. Maffei²;

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Studies were conducted in order to test a series of the monoethanolamine salts of *para*-substituted benzoic acids (MEASPBA), new compounds with biological activity as plant growth regulators and to evaluate the correspondence between the effects of different substituents on germination rate, root and shoot size, fresh and dry biomass, protein content, isocitrate lyase and catalase activity. The higher concentrations of the compounds inhibited the *Cucumis sativus* L. seed germination (exceptions were nitro- and amino- substituents), but lower concentrations had no effect or were stimulatory. The data showed that fresh biomass and root size decreased dramatically in seedlings incubated with compounds containing a halogen- (in order of its increasing atomic size) and nitro-substituents. The activity levels of isocitrate lyase (ICL, EC 4.1.3.1) and catalase (CAT, EC 1.11.1.6) in cucumber seedlings and their correlation with the above mentioned parameters are also discussed.

P1913. Phytochemical and Biological Investigation of *Dendrosicyos socotranus*N. A. Ali Awadh^{1,2}, U. Lindequist³, M. A. Al-fatemi¹, S. Hammod⁴;

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D. socotranus grows on the coastal plains and hills in Socotra island. It is the only tree in the *Curbitaceae* family. Dried powdered leaves were extracted in a Soxhlet apparatus successively with petroleum ether, dichloromethane, methanol, and cold water. Evaporation of the solvents was followed by drying in vacuo to provide crude extracts. The dried crude extracts were tested for antibacterial activity by using the modified disc diffusion method¹; for their cytotoxic effects on FL-cells by using the neutral red assay², and for their effects in the isolated rabbit ileum assay³. Phytochemical evaluation of plant extracts was performed using TLC. No antibacterial activity against (*S. aureus*1 (ATCC 29213), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) has been shown for the extracts till now. DM- and PE- extracts showed IC50 of 110 and 500 µg.

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P1914. Facilitation can be mediated by allelopathy in communities of arid-land plants

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In arid areas, plant coexistence is affected by different types of plant-plant interaction. In a field experiment, we found that facilitation by perennial *Artemisia aucheri* increased establishment for annual grass *Bromus tectorum*. Interaction was switched to competition and led to eventual death of *Artemisia*. Here we are arguing that facilitation can also be mediated by allelopathic effects. In a glasshouse experiment, *Bromus* seeds were grown in Petri dishes and irrigated with a solution containing 8 levels of *Artemisia* extracts. Furthermore *Bromus* seedlings were grown in pots containing 4 levels of *Artemisia* tissue powder plus field soil. Results indicate significant allelopathic effects of *Artemisia* on germination and seedling growth of *Bromus*, only at high concentration of extract and powder. Interestingly, under the same concentration of *Artemisia* powder, we found higher performance for *Bromus* in soils originated from *Artemisia* understory than bare field.

P1915. Study of Allelopathic potential of job's tear plant (*Coix lacryma-jobi* L.)

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Job's tear native perhaps to southeast Asia is a perennial grass and common weed in paddy fields in north of Iran. Because of the no information about allelopathic potential of the species, the experiment was conducted to determine the effect of the plant extract concentrations on germination, radicle and hypocotyle growth of test plants including; garden cress, lettuce and redroot pigweed. The collected seeds were cultivated in greenhouse and after four months growing the plants harvested. Vegetative plants body was dried and then ground. Concentrations with 2, 4 and 8% were prepared from extract and distilled water was used as control. The results showed that job's tear has high negative allelopathic potential. Test plants germination was reduced by increasing the concentrations with the exception of pigweed at 2%. Radicle and hypocotyle growth of the test plants were significantly reduced by increasing the concentrations with the exception of lettuce and pigweed hypocotyles at 2% in compare with control. In general radicle growth was more affected in compare with hypocotyle. Test plants had different sensitivity to job's tear allelopathic potential.

P1916. Restoration of riparian areas dominated by *Eucalyptus globulus*: allelopathic constraints

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Large areas of Portugal are covered by monocultures of the exotic *E. globulus*. The impact caused, in lotic systems, by eucalypt plantations may be minimized by the restoration of native riparian corridors. Herein we assess the allelopathic potential of eucalypt leaves and seeds against the deciduous *Alnus glutinosa* L.. Increasing concentrations of leaves leachates, obtained from artificial washing of leaves, were bioassayed for their phytotoxic effects on germination and seedling growth of alder. Low concentrations of eucalypt leachates significantly reduced radicle and aerial growth of the germinated seedlings. Eucalypt seeds incubation in agar culture medium significantly depressed alder but stimulated eucalypt germination. Germination rate and radicle growth seems to be negatively correlated with distance from the exudates origin.

We suggest that deciduous plantations in eucalypt streams riparian areas should be made in colder months to avoid high contents of leachates in the soil (due to the high summer input and impregnation with leachate rich stream water). Alder trees should be planted as saplings and at a distance from the eucalypt plantation.

P1917. Determination of selected phenolic compounds in common buckwheat (*Fagopyrum esculentum* Moench)J. Kalinova¹, N. Vrchotova², J. Triska²;¹University of South Bohemia, Faculty of Agriculture, Ceske Budejovice, Czech Republic, ²Laboratory of Analytical Chemistry, Institute of Landscape Ecology, Academy of Sciences of the Czech Republic, Ceske Budejovice, Czech Republic.

Buckwheat (*Fagopyrum esculentum* Moench) is known as good weed competitors. The phenolic compounds have wide biological activities and they are probably responsible for allelopathy potential of buckwheat.

The aim of this work was to identify and determine of selected the phenolic compounds in different parts of plant and their change during vegetation.

Common buckwheat (vyriety Emka) was grown in Ceske Budejovice (sand-loam brown soil) in year 2004. Lyophilized parts of plant (sampled three times during vegetation) were extracted by 90% methanol. Supernatants were analyzed by HPLC (DAD detector).

The highest content of quercetin, catechin and chlorogenic acid was determined in inflorescence. The highest content of derivatives of quercetin was in stems at the end of vegetation. Rutin culminated in leaves in flowerage period. Myricetin was identified only in stem.

The allelopathic activity of described compounds will be tested. This work was supported by grant GACR 521/03/D076 and AV0Z60870520.

P1918. Allelopathic potential of tea (*Camellia sinensis* (L.) Kuntze) on germination and growth of some plants species

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Effect of tea organs extract concentrations on germination, radicle and hypocotyle growth of garden cress, lettuce and redroot pigweed and golden foxtail weeds and effect of rates of their dried residues on leaf area, root, shoot and leaf dry weight of the weeds were studied. Concentrations and dried residue rates were consisting of 0, 2.5, 5 and 10% of organs extract and 0, 8, 16, 24 and 32g.kg⁻¹ respectively. The results showed that garden cress and redroot pigweed germination was reduced by all extracts of tea organs with the exception of leaf extract at 2.5%. Lettuce germination was inhibited by all extracts with the exception of leaf and fruit extracts at 2.5 and 5% concentrations respectively. Golden foxtail germination was also inhibited by all extracts with the exception of leaf and fruit extracts at 2.5% concentrations. Radicle and hypocotyle growth of studied plants were significantly reduced by all extracts of tea organs with the exception of lettuce hypocotyle at 2.5% concentration of leaf and fruit extracts. Weeds leaf area, root, shoot and leaf dry weight were significantly lower with tea organs residues especially at rates of 24 and 32 g.kg⁻¹ incorporation.

P1919. Effects of caffeine a toxic(allelopathic compound) on germination and growth of three wheat cultivarsL. Ziaehrhim^{1,2}, R. Khavarinejad², H. Fahimi²;¹Azad University, Kerman, Islamic Republic of Iran, ²Tehran Azad University Science and Research branch, Tehran, Islamic Republic of Iran.

In this study concentrations of 0, 50, 100, 200, 300 400 mg/l of caffeine used for germination and growth of 3 wheat cultivars. (higher concentrations inhibited germination). Time of first germination, germination percentage, wet and dry weight of roots and shoots (1st leaves), average length of roots and shoots and number of lateral roots estimated. Chlorophyll, protein and sugar content of both roots and shoots measured and finally activity of peroxidases measured in roots and shoots. Statistical analysis showed that caffeine caused delay in germination and germination percentage decreased. Dry and wet weight also showed deduction in root and shoot parts. Root and shoot length decreased more reduction in roots. Total amount of chlorophylls decreased, chl a reduced more than chl b. In concentration of 400mg/l amount of two chlorophylls are nearly equal. While starch content of seedlings not affected, soluble sugars slightly increased. Protein content of roots and shoots showed a slight decrease in high concentrations. Activity of antioxidant enzymes (peroxidases) in response to toxic situations strongly increased in roots more than shoots.

P1920. Impact of the waterweeds *Elodea* spp. on the performance of an aquatic herbivorous lepidopteran

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The submersed neophytes *Elodea canadensis* and *E. nuttallii* invaded many water bodies in Europe. Both species are well adapted to various environmental conditions and are allelopathically active against competing primary producers. Here we show that at least *E. nuttallii* is also chemically defended against important invertebrate herbivores; a trait that might further enhance the success of this plant. In our bioassays, waterweeds affected feeding, growth and survival of larvae of the lepidopteran *Acentria ephemerella*. These larvae preferably feed on various aquatic plants but only seldom on *E. canadensis* and never on *E. nuttallii*. Live plants influenced the larvae on different stages of their life cycle. Whereas newly hatched larvae mainly suffered from increased mortality, larvae after hibernation primarily faced growth inhibition. Extracts from *E. nuttallii* exhibited antifeedant properties, reduced growth and increased mortality of the larvae. Flavonoids present in *Elodea* might have accounted for the feeding deterrence, but did not affect survival of the larvae, indicating that chemical defence in *Elodea* is based on multiple compounds.

P1921. Two novel uses for *Arabidopsis thaliana* (L.) Heynh seeds.

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We report on two novel uses for the seeds of the cruciferous weed species, *Arabidopsis thaliana* (L.) Heynh. The first focuses on using the seeds as part of a set of standard target species in phytotoxic bioassays. Lettuce and other crop species have traditionally been used in that role. There is concern that they may not be sensitive enough and may not reflect what is actually occurring in the field. We tested seven known allelochemicals on *A. thaliana* seeds and compared them to lettuce seeds. Our study revealed that *A. thaliana* seeds were significantly more sensitive and useful in phytotoxic bioassays.

In a second study, we evaluated the effects of aerosol smoke on *A. thaliana* seeds. The results showed that smoke treatments ranging from 1-8 min significantly promoted rate of germination while 32-64 min inhibited it.

A. thaliana seeds appear to be ideal for phytotoxic studies and for those that focus on the response of germination to smoke treatments. The large body of knowledge already amassed for this species is an excellent resource for determining the effects of natural chemicals on seed germination and growth.

P1922. Study on the Allelopathic potential of bur parsly [*Tungenia latifolia* (L.)HOFF.]seed extracts collected from Roudbar, Iran.

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poster, Tonekabon, Islamic Republic of Iran.

Experiments were conducted to determine the effect of different concentrations of seed extracts of bur parsley on germination and growth of two aryland wheat (*Triticum aestivum* L.) cultivars (Rashid and Khalij) and bur parsley seeds. Polyethylen glycol (PEG) was used to determine the influence of osmotic potential on the bioassay materials. Bur parsley seed extract concentrations of 60, 80 and 100% of original strength reduced the germination of two wheat cultivars after three days. After seven days, germination of Rashid wheat seeds was significantly reduced by using seed extract concentrations of 40, 60, 80 and 100% and germination of Khalij wheat seeds was significantly reduced by using seed extract concentration of 80 and 100% seed extract concentration also reduced coleoptile and radicle lengths in the two wheat cultivars. Bur parsley seeds were not sensitive to the lower concentration of their own extract. The results of this study show the allelopathic potential of bur parsley seed extracts on the two dryland wheat cultivars.

P1923. The alkaloid liriodenine during germination of *Annona diversifolia* Saff.

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Annonaceae plants produce a series alkaloids, like liriodenine an oxoaporphine alkaloid that plays an important role in the plants that produce it. This research was made in *Annona diversifolia* Saff for know the first stages of liriodenine production during the development. The quantification was done by HPLC in the organs and tissues from the newly collected seed, with two and ten days of imbibition, when the radicle emerged in the stage of seedling. According with the results the liriodenine is not imported from the mother plant and is not a product of the embryogenesis, because it appears for the first time in the endosperm at the tenth day of imbibition, remaining exclusively in this place in spite of the development of one cm radicle. Once the seedling stage has initiated it the liriodenine can be found in roots and stems and it is absent from the cotyledon leaves and from the true leaves. Due to this, its biosynthesis is organ specific and seems to be controlled by early development programs in the plant that represent an important biological function, perhaps in the defense mechanism of the plant.

P1924. Presence of lichen compounds in the butterfly *Talica niseus* (Lycaenidae)

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During our studies on Sri Lankan lichens, we discovered the sequestration of aromatic lichen products such as usnic acid and atranorin the triterpenoid zeorin and a long chain fatty acid ester belonging to the crustaceous lichen *Leproloma sipmanianum* Kummerl & Leukert by the butterfly *Talica niseus* Guerin (red pierrot) in Beragala, Uva province, Sri Lanka. *T. niseus* was found flying close to the extensive lichen thallus, growing on road side proterozoic rocks of gneiss and quartz schist which are exposed at road edges, and roosting on it periodically. The presence of these compounds in the butterfly was confirmed by comparative TLC and HPLC studies with authentic lichen substances isolated from the lichen *L. sipmanianum*. The presence of lichen compounds in both the larvae and larval waste collected from Beragala suggests that the adult butterfly was accumulating the lichen compounds through the larvae and was using the lichen as an alternative food source, probably when the natural food source *Bryophyllum calycinum* is not plentiful.

P1925. Comparison of defences induced by piercing-sucking and chewing herbivores in *Medicago truncatula*

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The modes of chemical defence in plants can basically be divided in induced and constitutive ones. The former can further be classified in indirect and direct defensive mechanisms.

Induced phytochemicals in *Medicago truncatula* were investigated using the defence against two arthropod pests (*Spodoptera littoralis* and *Tetranychus urticae*) with different feeding manners as example.

As component of indirect defence a sizeable variety of volatiles is released, particularly of sesquiterpenoids. Regarding direct defence, the deposition of phenolics at the wounding sites was observed with a similar pattern as described for pathogen attack. Moreover, the accumulation of reactive oxygen species was detected adjacent to the wounds.

These reactions are at least in part mediated by the phytohormones salicylate and jasmonate. Both, the pattern and the time course of their accumulation clearly differs depending on the inducing herbivores.

P1926. Cyanogenesis of Lima Bean (*Phaseolus lunatus* L.): Intraspecific and Ontogenetic Variability

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Cyanogenesis is a process by which hydrogen cyanide (HCN) is released from endogenous cyanide-containing compounds as reaction to cell or tissue disintegration.

We found substantial variation of the cyanogenic potential (HCNp; the total amount of HCN which can be released from a given plant tissue), specific α -glucosidase activity and the resulting cyanogenic capacity (HCNc; the release of HCN per time unit) by screening for

cyanogenic properties of 14 lines of *P. lunatus*. In addition to this intraspecific variability a distinct ontogenetic variability of cyanogenic properties in leaf material occurred.

Plant cyanogenesis is considered to act as a constitutive plant defence but our studies on the obligate cyanogenic Lima bean let assume a plastic character of cyanogenic features in quantitative aspects. Analysis of Lima bean plants infested with two-spotted spider mites (*Tetranychus urticae* Koch) and Mexican bean beetles (*Epilachna varivestis* Mulsant) revealed an increase of specific α -glucosidase activity within the damaged leaf tissue.

P1927. Unprecedented oxylipin pathways in the moss *Physcomitrella patens*: Isolation and characterization of a new multifunctional lipoxygenase with lyase cleaving activity.

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The moss *Physcomitrella patens* was chosen as model system to study plant lipid metabolism since it harbors a wide range of different polyunsaturated fatty acids. Here oxylipin pathways responsible for volatile formation in this moss were investigated. Upon cell damage (*R*)-1-octen-3-ol, 2-octen-1-ol and (5*Z*,8*Z*,10*E*)-12-oxo-dodecatrienoic acid (12-ODTE) as well as (2*E*)-nonenal and 11-oxoundeca-5,9-dienoic acid (11-OUDE) were identified as major oxylipins. Most interestingly all volatile oxylipins turned out to be cleavage products of arachidonic acid.

We isolated a lipoxygenase (LOX) with yet unknown catalytic properties. The enzyme shows high sequence homology to linoleate 13-LOX from plants. In addition, it was characterized as an arachidonate 12-LOX with preferences against C20 polyunsaturated fatty acids. This enzyme exhibits a high hydroperoxidase- and a yet not known fatty acid cleavage activity. Thus the action of this multifunctional LOX directly led to formation of the identified unsaturated alcohols and 12-ODTE when arachidonic acid was used as substrate. Interestingly, (2*E*)-nonenal and 11-OUDE do not belong to its product spectrum.

P1928. Activity of Acetylcholinesterase in larvae an adult of the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) using diets containing Burseraceae plants

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Acetylcholinesterase, a serine hydrolase, catalyzes the breakdown of the neurotransmitter acetylcholine into acetate and choline. The enzymatic inhibition of the acetylcholinesterase has been used in order to test the effects of diverse compounds upon insect physiology. In the present work, we evaluated separately whether an artificial diet containing dry plants or methanolic extracts at different concentrations of *Bursera lancifolia*, *B. aleoxyllon*, *B. grandifolia*, *B. aptera* and *B. longipes* widely distributed in Mexico, has an effect in acetylcholinesterase inhibition of larvae and adult of *Spodoptera frugiperda*. Results obtained indicate that Acetylcholinesterase inhibition was about 70-90 % in larvae and 5-30% in adults with all Burseraceae species. *B. lancifolia* was the one with main effect (98% in larvae and 30% in adults). These data suggest that some compounds of these plants, possibly terpenics, have anticholinesterase activity in this insect-plague which represent a devastating highly cost crops damages for our country.

P1929. Neoplastic formations on trees: host plant tissues are affected

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In order to test a hypothesis that neoplastic formation on trees induced by arthropods affects biochemical characteristics of both newly formed galls as well as host plant tissues, biochemical characteristics with a possible adaptive role were determined in nine gall former - host tree combinations. Photosynthetic pigments, extractable protein content, and oxidative enzyme activities were determined in gall tissues, leaf tissues of galled leaves, and leaves on ungalled tree branches. Neoplastic tissues were characterized by a low content of pigments, decreased chlorophyll a/b ratio, lower extractable protein content, and decreased activities of

peroxidase and polyphenol oxidase as compared with ungalled host leaf tissues. In galled leaves or in leaves adjacent to galls increased level of peroxidase activity was found. In several gall inducer - host plant combinations, galled host plant tissues contained increased activity of polyphenol oxidase as well. The presented data reflect long-term systemic effects of neoplasmic formation on host-tree physiology suggesting that gall inducers affect potential adaptive responses of host plants.

P1930. Cytokinins in the gall forming wasp *Diplolepis rosae* and its galls on *Rosa canina*

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Attempts to determine the chemical signals of the gall-inducing insect, that initiate the gall formation on host plant and its development, resulted up to date in an identification of a number of different compounds that appear to be involved in cecidogenesis. Among them, elevated amounts of naturally occurring plant hormones were found in galls, including auxins and cytokinins. In the present work the levels of 39 cytokinins in galls induced by wasp *Diplolepis rosae* on *Rosa canina* were quantified and identified, with iP, iPR and BAP to be the most abundant. In larvae of *D. rosae*, 11 of the present cytokinins showed significant variations in time course during the wasp lifecycle. The most dramatic changes were found in iP and iPR levels. Significant cytokinin levels were found in quiescent larvae and surprisingly also in adult wasps. Physiological explanation for that finding evokes speculations on the actual origin of cytokinins in gall-forming insect and their physiological role.

P1931. Distribution and quality of chemical cues for a herbivores' host plant recognition

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The first contact zone between an insect and its host plant is the plant surface. However, epicuticular leaf waxes of *Nasturtium officinale* (Brassicaceae) were shown to lack feeding stimulatory activity for the mustard leaf beetle *Phaedon cochleariae*. Only by damaging the wax layer, the beetles might receive information on the host plant quality. Thus, in these plants, the leaf wax coverage can be regarded as defensive shield against specialist herbivores. Nevertheless, extracts of *N. officinale* leaves evoked strong feeding activity. In bioassays, the most characteristic secondary plant compounds of Brassicaceae, glucosinolates, did not stimulate feeding. Therefore, analyses of fractionated host plant extracts and corresponding bioassays led to the isolation of further stimulating plant compounds. Potential additive or synergistic effects of these plant metabolites from inner leaf tissue on the feeding behaviour of *P. cochleariae* were investigated.

P1932. Insecticidal activity of deoxypodophyllotoxin, isolated from *Juniperus sabina* L., and related lignans against larvae of *Pieris rapae* L.

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A petroleum ether extract of *Juniperus sabina* L was found to show insecticidal activity against larvae of *Pieris rapae* L. From the extract, an insecticidal compound was isolated by bioassay-guided fractionation. The compound was identified as deoxypodophyllotoxin (1). In bioassays, 1 showed antifeedant activity against larvae of *P. rapae* at 0.05-1.00 g litre⁻¹ and its AFC₅₀ (concentration for 50% antifeedant activity) values at 12 and 48 h were 0.170 and 0.060 g litre⁻¹, respectively. In that concentration range, all treated insects died within 48 h after treatment and compound 1 showed delayed insecticidal activity. At 0.015-0.100 g litre⁻¹, 1 showed insecticidal activity, with an LC₅₀ of 0.020 g litre⁻¹. The related compound deoxypicropodophyllotoxin (2), showed lower antifeedant and insecticidal activities than 1 in bioassay. This indicated that the *trans*-lactone ring is an important moiety for enhancing activity in these compounds. Comparison of the insecticidal activities of 1 and another related compound, podophyllotoxin (3), suggested that varying the substituent at C-4 is an exciting possibility for synthesizing more potent analogues.

P1933. The effect of root exudates on plant-fungus-interactions in the tomato rhizosphere

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The stimulation of plant pathogens by root exudates is an integral part of the concept of inoculum potential. Propagules of several plant pathogenic fungi have been shown to germinate in the presence of root exudates, but as reviewed by Nelson (1991) little is known about the nature of specific exudate molecules initiating fungal growth. In our study we assessed spore germination and mycelial development of the tomato pathogens *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* in the presence of root exudates and several flavonoids. We could show that tomato root exudates contained a major amount of phenolic compounds which affect the development of *F. oxysporum* in the course of plant development. The possible involvement of flavonoids in the fungal development will be discussed.

Nelson, E.B. 1991: Exudate molecules initiating fungal response to seed and roots. pp 197-209 in: The rhizosphere and plant growth. D. L. Keister & P. B Cregan, eds. Kluwer, Dordrecht.

P1934. Evaluation of *Balanites aegyptiaca* Del. (*Zygophyllaceae*) kernel cake saponins and their larvicidal effect against *Culex pipiens* mosquito larvae

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Balanites aegyptiaca Del. is a drought-tolerant desert tree native to Africa, the Middle East and South Asia. Although various plant parts are widely used in folk remedies (whose activities may be attributed to the saponin constituents), this plant remains one of the world's most neglected species. To widen the uses of this plant - and hence to aid in its domestication - a methanolic extract of *B. aegyptiaca* kernel cake after oil extraction was analyzed phytochemically (LC-MS, NMR and colorimetry) for saponin constituents and tested for its larvicidal effect on *Culex pipiens* mosquito larvae. The kernel cake contained a large amount of saponins, having the sugar moiety attached in positions C-3 and C-26 and diosgenin as the aglycone. The main steroid saponin was 26-(O-⁻-D-glucopyranosyl)-22-O-methylfurost-5-ene-3, 26 diol 3-O-⁻-D-xylopyranosyl-(1 3)-⁻-D-glucopyranosyl-(1 4) [-L-rhamnopyranosyl-(1 2)]-⁻-D-glucopyranoside. The high larvicidal activity of the extract against *C. pipiens* larvae (LC₅₀ 76 ppm and LC₉₀ 545 ppm in 24 h) indicates that the kernel cake can be used as an effective larvicide (the saponins being the main active agents).

P1935. The effects of herbivore damage and elevated ozone concentration on the volatile terpenoids produced by two hybrid aspen (*Populus tremula x tremuloides*) clones

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The terpenoids produced by two clones of hybrid aspen in response to herbivory by leaf weevils (*Phyllobius argentatus*), and larvae of the autumnal moth (*Epirrita autumnata*), have been studied in field plots running for two years under ambient and enhanced ozone levels. The volatile organic compounds produced by the differentially treated aspen clones were collected from either intact tree material in the field, or from cut twigs transported to the laboratory. Data was collected during the summer of 2004, and it is planned to increase the data set in 2005 using the field collection protocol. Tentative analysis of the data collected in 2004 suggests that isoprene emissions are not influenced by herbivore damage, but that there is some induction of monoterpenes, sesquiterpenes and homoterpenes, with particular induction of the monoterpene (cis)-beta-ocimene. A doubling of the ambient ozone concentration did not appear to induce terpenoid emission, but there was some clonal variation in the emission quantities of the homoterpene (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT). The relative importance of ozone and herbivore damage on VOC induction will be discussed.

P1936. Future ozone-enriched environments: Can they affect plant-insect interactions?

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Terpenes play an important role in chemical communication between plants and insects. They react with ozone (O₃), tropospheric levels of which are predicted to rise. This is likely to affect herbivore induced plant volatile blends and influence plant insect interactions. We addressed 1) how O₃ alters the blend of terpenes emitted by cabbage plants, 2) how O₃ concentrations are influenced by reaction with phytogetic terpenes and 3) if secondary organic aerosols -SOA are detectable in the oxidation of phytogetic terpenes. White cabbage (*Brassica oleracea* cv Lennox) plants were treated with methyl jasmonate or infested with *Plutella xylostella* and placed in a 22-liter glass desiccator. O₃ was conducted onto plants at various levels. The headspace volatiles were collected. In a second experiment, to avoid loss of O₃ on plant surfaces, volatiles were conducted into a FEP Teflon tube chamber where O₃ flow was adjusted and monitored at the inlet and exit. Plant volatiles were collected before and after the reaction chamber and analyzed by GC-MS. Particle size distributions were measured at the end of the reaction chamber to analyze SOA. Preliminary results are presented.

P1937. Anatomy and histochemistry of leaves from *Omphalea oleifera* (Euphorbiaceae) and its relationships with the herbivore *Urania fulgens* (Uranidae).

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Herbivore feeding by rainforest insects includes underground parts and plant fluids. The *Omphalea oleifera* (Euphorbiaceae), present in Los Tuxtlas Mexico; is subject of intense herbivory. In the present work, we studied anatomy and qualitative and quantitative aspects their histochemistry leaves. The calcium oxalate crystals (druses and prismatic) are abundant in leaf's adaxial and abaxial epidermis. Laticiferous containing insoluble polysaccharides, phenols and lipids, are associated to vascular tissue and are present in all venation. Lignin deposits were detected around harmed areas by herbivore activity, which suggest a healing to avoid infections. Calcium oxalate crystals and the percentage of lignification found in leaves tissue were 8-10%; as well as laticiferous components, could participate reducing herbivory. Ecological studies, have shown that *Urania fulgens* feed adult plants, juvenile and seedlings being totally defoliated by this diurnal moth larvae. Thereafter it is of interest to study *U. fulgens* in order to know the physiological mechanisms by means this insect digest this plant, being considered as a specialist for *O. oleifera*.

P1938. Possible role of secondary metabolites in the host choice by codling moth larvae (*Cydia pomonella*)

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The question how fruit miners select the appropriate host is central in understanding the biology of codling moth, *Cydia pomonella* L., the most important insect pest in pome fruit production. In this study, methanolic extracts of leaves of *Tanacetum vulgare* and of inflorescences of *Erigeron annuus* were found to be feeding deterrent against newly hatched larvae. Bioassay-guided fractionation with dual choice tests on artificial substrate indicated that the most lipophilic fractions of both extracts contained the active compounds. Gas chromatography/mass spectrometry analyses revealed a group of sesquiterpenes as main constituents of the active fraction of the *E. annuus* extract. One of these, identified tentatively as a sesquiterpene with an aromadendrane skeleton by library comparison, deserves special attention, since a compound with identical spectrum is also present in the active fraction of *T. vulgare*. These findings suggest that secondary metabolites may interfere with host plant acceptance of codling moth larvae besides that of adults.

P1939. Biological activities of *Stemona* species

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Stemonaceae represent a rather isolated family within the Monocotyledons consisting of 3 genera and about 32 species. *Stemona* is the largest genus with about 25 species occurring as subshrubs or twining herbs mostly with perennial tuberous roots. Since the roots of several species are widely used as insecticides and for medicinal purposes, they are offered for sale on local markets in Thailand, China and Japan. However, because of the similar shape of the fleshy tuberous roots, quality control clearly showed that confusions with different species and even with representatives from other plant families are common. This uncertainty in purchasing appropriate plant material and unsolved taxonomic problems at the species level have led to confusions in chemical and pharmaceutical literature.

Stemona is characterized by family-specific alkaloids, showing an unusual molecular architecture with a pyrrolo[1,2-a]azepine nucleus or a pyrido[1,2-a]azepine nucleus. Moreover, the accumulation of stilbenoids is a typical chemical trend of many *Stemona* species. To contribute to a better understanding of the bioactive capacities of *Stemona* chemicals, we present antioxidative, insecticidal and cytotoxic activities from selected species and geographical provenances.

P1940. The importance of being red when young: field evidence that anthocyanins in juvenile leaves of *Quercus coccifera* afford camouflage against insect consumers but no protection against excess light

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We have tested two hypotheses for the function of red anthocyanins transiently appearing in young leaves i.e. the photoprotective hypothesis against excess light and the handicap signal against herbivory. We took advantage of the intraspecific variation in anthocyanin levels in young leaves of *Quercus coccifera*. PSII photochemical efficiencies measured in the field under white or red light (which by-passes anthocyanins) were not indicative of a photoprotective role. Yet, red phenotypes suffered considerably lower insect attacks and contained higher levels of deterring phenolics. We suggest that an anthocyanin screen may not be photosynthetically important in young, thin low chlorophyll leaves, where the attenuated green light may not be important for chlorophyll excitation. Yet, the leveling of reflectance in the 400-570 nm band makes red leaves less discernible to insect herbivores. Even after accidental approach, overconsumption is discouraged by high phenolic levels.

P1941. Infection process in sclerotial dieback of *Cryptomeria japonica* - competitive fungi in needle insect galls-

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Scolicosporium sp., the causal pathogen of sclerotial dieback of Japanese cedar (*Cryptomeria japonica*), was frequently isolated from needle galls associated with the gall midge insect *Contarinia inouyei* Mani. It was hypothesized that the pathogen establishes in needle tissue by taking advantage of the microenvironment conferred by the galls. The purpose of this study was to identify the gall ENDOPHYTES and relate these to the presence of *Scolicosporium*. Isolation tissues were separated into four categories: (1) needle tissue before gall formation; (2) incipient gall with larva; (3) necrotic gall with larva; and (4) necrotic gall post larval exit. *Pestalotiopsis* sp., *Rhinoctadiella* sp. and *Cladosporium* sp. were frequently isolated from (1) whereas *Scolicosporium* sp. was absent. However, *Scolicosporium* sp. was isolated from (2), (3) and (4) together with *Pestalotiopsis* sp., *Rhinoctadiella* sp. and *Cladosporium* sp. This investigation strongly suggested that *Scolicosporium* sp. first establishes at the incipient gall with included larva stage and that *Pestalotiopsis* sp., *Rhinoctadiella* sp. and *Cladosporium* sp. were the predominant ENDOPHYTES.

P1942. Carboxylesterase-like proteins dehydrate 2-hydroxyisoflavanone in leguminous isoflavone biosynthesis: implication for the recruitment of enzymes from primary to secondary metabolism

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Isoflavonoids are ecophysiologicaly-active natural products of the leguminous plants. Isoflavones are early products of the pathway and synthesized by the dehydration of 2-hydroxyisoflavanones, the

products of the P450 isoflavonoid synthase. cDNAs of 2-hydroxyisoflavanone dehydratases (HIDs) producing 4'-methoxy- and 4'-hydroxy-isoflavones were cloned from leguminous plants *Glycyrrhiza echinata* and soybean, respectively. The substrate specificity of HIDs agreed with the profiles of isoflavonoids produced by each plant species. Surprisingly, HIDs were found to be the members of carboxylesterase family to which many plant proteins involved in defense responses but with unknown catalytic activity belong. The amino acid residues composing the oxyanion hole and catalytic triad are important for both HID and a faint ester hydrolysis activity. HIDs must have been recruited from enzymes of the primary metabolism during evolution. Homology modeling and site-directed mutagenesis of HIDs are in progress to elucidate the structural requirements for the different activities.

P1943. Identification of anthocyanidin synthase gene promoters in apples

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The accumulation of anthocyanins in apple fruit skin is genetically determined and influenced by environmental factors including temperature and light. We have shown that the expression level of the anthocyanin biosynthetic genes in the fruit skin of 'Akane' (a well-colored cultivar) at the stage of ripening was higher than that in 'Tsugaru' (a poorly-colored cultivar), and that the expression increased under low temperature and UV-B conditions in both cultivars (submitted). In particular, the expression of *anthocyanidin synthase* (*ANS*) gene was remarkably induced under these conditions. Southern blot analysis indicated the polymorphism in the genomic structure of *ANS* genes in 'Akane' and 'Tsugaru'. We isolated the five clones containing *ANS* gene from the 'Tsugaru' genomic library. The nucleotide sequences of the coding region of the five clones were almost identical, while those of the promoter region of the five clones were different from each other. Isolation of *ANS* genes from the 'Akane' genomic library is currently being conducted to compare the nucleotide sequences of the promoter region that is responsible for regulation of the gene expression.

P1944. Evolution of benzoic acid-specific type III polyketide synthases

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Type III polyketide synthases (PKSs) catalyze the iterative condensation of a starter substrate with malonyl-CoAs and the subsequent cyclization of the intermediate polyketide. Variation of either the starter substrate or the number of condensations and the type of cyclization leads to an impressive array of secondary products. Benzophenone synthase (BPS) and biphenyl synthase (BIS) are PKSs which prefer benzoyl-CoA to form identical linear tetraketides. While this intermediate is cyclized by BPS via an intramolecular Claisen condensation, BIS catalyzes an intramolecular aldol condensation and decarboxylative elimination of the terminal carboxyl group. The enzymes share 54% amino acid sequence identity. In a phylogenetic tree, BPS and BIS group together with PKSs which functionally differ from the ubiquitous PKS, chalcone synthase (CHS). CHSs form an individual cluster. CHS-derived BPS and BIS homology models guided the generation of site-directed mutants.

P1945. 4-Coumarate:CoA Ligase in the Moss *Physcomitrella patens*

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4-Coumarate:CoA Ligase is a central enzyme of general phenylpropanoid metabolism and provides CoA thioesters of ring-substituted cinnamic acids as precursors for a large variety of important plant secondary products such as lignin, flavonoids, or phytoalexins. The existence of 4CL isoforms with different substrate specificities and their phylogenetic reconstructions indicate, that higher plant 4CLs can be classified into two large, evolutionary ancient classes. To clarify the evolutionary origin, we analyzed 4CL in the moss, *Physcomitrella patens*. Two structurally different 4CL genes, *Pp4CL1* and *Pp4CL2*, were identified. The proteins show an identity of about 62% when compared with

higher plant 4CLs. However, the substrate specificity of *Pp4CL1* is distinctly different from that of higher plant 4CLs. The recombinant enzyme, produced in *E. coli*, efficiently converted cinnamate to the corresponding thioester, while cinnamate is a very poor substrate for higher plant 4CLs. This suggests an important role for cinnamate as a physiological substrate in *P. patens*.

P1946. Polymorphisms of cannabinoid synthase gene in *Cannabis sativa*.

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Cannabis (*Cannabis sativa* L.), one of the oldest cultivated plants, has been grown worldwide for thousands of years for its fiber and seed oil. In addition, the dried flowering tops and leaves are used as the narcotics marijuana and hashish. In the present study, cannabinoid (narcotic chemical substances) contents and DNA polymorphisms of eight different strains of *C. sativa* were analyzed. There were two chemotypes; "chemotype I" with high delta-9-tetrahydrocannabinolic acid (THCA) content and low cannabidiolic acid (CBDA) content, and "chemotype II" with equal content of THCA and CBDA. Genomic DNA sequence polymorphisms in the THCA synthase gene were studied. The defined sequences of the THCA synthase gene in eight strains were classified into three types as "[T][G][T]-type", "[T][A][T]-type", and "[A][A][A]-type". All genomic polymorphisms observed in the present study seemed to be synonymous substitution, not affected amino acid sequences, and also the enzymatic activity of THCA synthase.

P1947. Polyphyletic origin of pyrrolizidine alkaloids. Differential tissue expression of homospermidine synthase.

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The evolution of pathways within plant secondary metabolism has been studied by using the pyrrolizidine alkaloids (PAs) as a model system. PAs are constitutively produced by plants as a defense against herbivores. The occurrence of PAs is restricted to certain unrelated families within the angiosperms. Homospermidine synthase (HSS), the first specific enzyme in the biosynthesis of the necine base moiety of PAs, was originally recruited from deoxyhypusine synthase (DHS), an enzyme involved in the posttranslational activation of the eukaryotic initiation factor 5A. Recently, this gene recruitment has been shown to have occurred several times independently within the angiosperms and even twice within the Asteraceae. Immunolocalization experiments have shown that, within these two PA-producing tribes of the Asteraceae, viz. Senecioneae and Eupatorieae, HSS is expressed differently, despite catalyzing the same step in PA biosynthesis.

P1948. Caffeine biosynthetic enzymes are closely homologous each other in coffee.

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In coffee plant, caffeine is synthesized from xanthosine via a pathway that has three S-adenosylmethionine-dependent methylation steps. We identified and characterized respectively genes encoding enzymes for these three methylation steps of caffeine biosynthesis. Although these genes, named CmXRS1, CTS1, CTS2 and CCS1, are closely homologous each other, these enzyme specificities are strictly divided. Caffeine biosynthesis is presumed to begin with CmXRS1 converting xanthosine to 7-methylxanthosine. CmXRS1 can catalyze only the first methylation step. CTS1 and CTS2 only catalyze the second methylation step that is the conversion from 7-methylxanthine to theobromine, and do not have 1-N-methylation activity that is third methylation step. CCS1, like tea TCS1, can catalyze the second and the third methylation steps. The structure/function relationships of these N-methyltransferases that are involved in caffeine synthesis are discussed with YOU!

P1949. Isolation and functional analysis of caffeine synthase and related methyltransferases in plants

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Caffeine synthase (CS) in tea (*Camellia sinensis*) leaves is an S-adenosylmethionine (SAM) dependent N-methyltransferase that catalyzes the last two methylation steps in the caffeine biosynthetic pathway. CS methylates N-3 and N-1 positions of purine ring and work as a key enzyme in caffeine biosynthesis. We purified CS and isolated cDNA, named TCS1. High levels of transcripts of TCS1 were detected in developing leaves and much lower amounts were present in old leaves. These expression patterns mirror the distribution of caffeine synthase in tea leaves. Furthermore, we isolated TCS homologous gene from theobromine (3,7-dimethylxanthine)-accumulating plants, *Camellia irrawadiensis* and *Theobroma cacao*, in order to compare the function of TCS homologous gene. Recombinant enzymes from these TCS homologous genes possess 3-N- but not 1-N-methylation activity. The accumulation of purine alkaloids is therefore, depend on N-methyltransferase substrate specificity.

P1950. Growth and production optimization of tropane alkaloids in *Datura stramonium* cell suspension culture

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Tropane alkaloids play vital roles in pharmaceutical industries. This work is conducted to optimize the physicochemical factors affecting the growth and production of tropane alkaloids in *Datura stramonium* (Solanaceae). Cell suspension was prepared from semicircular calli of leaf explants in MS medium. Results showed that at 30 and 40 g/lit glucose and 20 and 40 g/lit sucrose concentrations the highest production of alkaloid and biomass will be obtained respectively. High concentration of nitrate led to reduction of the alkaloids production. The best concentration of potassium nitrate was 9.4mM for alkaloids and 37.6mM for biomass. Also it was evidenced that the optimized concentration of ammonium nitrate for alkaloids production was 10.3mM, and for the biomass was 41.22mM. The study of calcium chloride indicated that the best concentration for the growth and production of alkaloids was 7.92mM. Study on the effects of different temperature revealed that the best condition for the highest production of alkaloids was at 20°C and that of the most biomass production was at 25°C.

P1951. Molecular analyses of a glucosinolate polymorphism in *Barbarea vulgaris* (Brassicaceae).

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The capacity of plants to defend themselves against phytophages heavily depends on the quantity and quality of secondary metabolites. For crucifers the most well-known defense compounds are glucosinolates. In Dutch *Barbarea vulgaris* populations, we have found different frequencies of two naturally occurring chemotypes: one produces mainly 2-phenylethylglucosinolate (gluconasturtiin) whereas the other produces mainly the hydroxylated form, S-2-OH-2-phenylethylglucosinolate (glucobarbarin). Due to this single 2-hydroxylation, the glucosinolates and their hydrolysis products may have a different toxicity.

We found that the heritability of this polymorphism is determined by a single gene with a dominant allele for glucobarbarin and a recessive allele for gluconasturtiin. Based on a similar 2-hydroxylation enzyme found in *A. thaliana*, we hypothesize that the hydroxylation of gluconasturtiin is performed by a 2-oxoacid dependent dioxygenase (2ODD). We use degenerate primers based on that 2ODD in *A. thaliana*, and homologues of that gene in other plants, to identify the enzyme responsible for differences in glucosinolates in *B. vulgaris*.

P1952. Characterization of dihydrodipicolinate synthase in opaque and floury maize endosperm mutants

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Maize is an important source of protein for human and livestock. However, the nutritional quality is not adequate, due to the lack of the essential amino acids lysine and tryptophan in the seed. Dihydrodipicolinate synthase (DHDPS) is the key regulatory

enzyme which controls the rate of lysine synthesis in the aspartate pathway, catalyzing the condensation of pyruvate and aspartate - semialdehyde. We have studied DHDPS activity in the endosperms of four different lines of maize with single opaque and floury mutations, which have been shown to contain higher concentrations of lysine: Oh43 (WT), o1, o2, f11 and f12; W22 (WT) o10, o11 and o13; B37 (WT) and o7; B77/79 (WT) and o5. The developing endosperms were harvested at 16, 20 and 24 days after pollination (DAP). DHDPS activity increased 20 and 24 DAP and was shown to vary considerably in the mutants. The f12 and o13 mutants exhibited the highest and lowest activities at 20 DAP, respectively. Lysine inhibited DHDPS activity at low concentrations, but the amount varied depending on the mutation, indicating an altered regulation of DHDPS activity. Financial support by FAPESP and CNPq.

P1953. Both steroidal saponogenin and steroidal alkaloid are glycosylated by a single glycosyltransferase

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We succeeded in cloning a cDNA for steroid glycosyltransferase from *Solanum aculeatissimum*, for the first time. The Solanaceae contain steroid saponins with diverse biological and pharmacological activities. Glycosylation is the final step in the biosynthesis of saponins, and their sugar chain structure is considered important for their activities. After screening gene fragments for putative glycosyltransferases (GTs) from several *Solanum* sp., three candidates for full-length cDNAs were obtained from *S. aculeatissimum*. Recombinant GTs were expressed in *E. coli* as GST fusion proteins. One of the GTs, named SaGT4A, showed sugar acceptor specificity for some steroidal saponogenins, as well as spirostane-type alkaloids. Gene expression analysis revealed that the accumulation of SaGT4A transcripts showed a unique response to wounding stress indicating the involvement of SaGT4A in plant defense.

P1954. The root organogenesis and indole alkaloid production in Leaf _ stalk of *catharanthus roseus*

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Periwinkle (*Catharanthus roseus* G. Don) is a unique species containing dimeric indole alkaloids of vinblastine and vincristine that have become highly valued drugs in cancer chemotherapy due to their potent antitumor activity against various leukemias, Hodgkin's disease and solid tumors. Tissue cultures originating from leaf_stalk of *C. roseus* were initiated under various concentration of NAA with Kin treatments and subcultured to give the callus, root or shoot regeneration. The different calli and the regenerated roots were analyzed by TLC and HPLC with respect to the indole alkaloids producing capacity. The root calli cultures were induced from leaf_stalk of *C. roseus* in the presence of 0.1mg/l NAA with 0.1, 5, 10 and 20mg/l Kin. Of the 16 groups cultured, the MS with NAA=Kin=0.1mg/l of group had the highest vindoline, catharanthine, vincristine and root organogenesis capacity. In this roots were produced the highest vincristine (6fold), catharanthine (10fold), serpentine (125fold), vinblastine (20fold), vindoline (0.5fold) and ajmalicine (0.34fold) compared to that in the leaf_stalk of intact plants.

P1955. Composition of the Essential Oil within Summer Savory (*Satureja hortensis* L., Lamiaceae) from Syria Based on SPME Single Gland Analysis

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The essential oil of Lamiaceae is produced in oil glands on the leaf epidermis and stored in the glands subcuticular space. An interesting approach to better understand the variability of the essential oil composition within a plant is the analysis of single oil glands.

Oil glands from summer savory (*Satureja hortensis*) (petals, calyxes, young, medium and old leaves) were analysed by sampling the content of each oil gland with a SPME-fibre.

The main compound carvacrol was highest in the petals (85%). The calyx showed intermediate values of 75% while carvacrol was equal in all three leaf positions (young, medium and old leaf) with

58%. -Terpinen increased from 9% in the calyx to 30% in the leaves.

The lanceolate leaves were divided into four sections starting from the leaf base (no. 1) to the leaf top (no. 4). The content of carvacrol increased from 56% on sector no. 1 to 66% in sector no. 2 and decreased then steadily down to 53% in sector no. 4. -Terpinene showed the opposite trend with a decrease from 32% (sector no. 1) to 25% (sector no. 2) and a steady increase to 36% in sector no. 4.

P1956. Biosynthesis of Isoprenoids in Tobacco BY-2 Cells

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For our studies we have chosen tobacco Bright Yellow-2 (TB-2) cells, which are characterized by high metabolic flux rates. The MVA pathway, mainly leading to the formation of sterols, but also of the side chain of mitochondrial ubiquinone, occurs in the cytoplasm and can be affected by mevlinolin, but also by compounds that block reactions upstream or downstream of the rate-limiting HMG-CoA reductase (HMGR) step. Some of those compounds act as cell cycle inhibitors. It seems that the de novo synthesis of a product downstream from MVA and possibly close to some end product (sterol or intermediate) is essential for feedback suppression of HMGR. We can visualize in TB-2 cells the isoprenylation of GFP, fused with a protein domain bearing a CaaX prenylation motif. Absence of isoprenylation leads to migration of modified GFP mainly into the nuclei instead of targeting to the plasma membrane. The system recognizes effects exerted by inhibitors, also of the alternative plastid isoprenoid (methylerythritose phosphate = MEP) pathway, or by intermediates potentially piling up. It also provides a means for the study of metabolic cross-talk between the MVA and the MEP pathway.

P1957. Quantitative study of epithelial cells in *Carum carvi* fruit secretory canals

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C. carvi fruits contain 3-7% of essential oil with carvone and limonene as main compounds. The essential oil is synthesized in fruit secretory canals of two types: flat and rounded. Area of epithelial cells in flat canals (FCC) is 47 μm^2 and that of epithelial cells in round canals (RCC) is 25 μm^2 . Endoplasmic reticulum (ER) in FCC is scanty, cisternal, in RCC - abundant, tubular. Mitochondria in FCC are numerous (12, number per cell cross section is given), small (average area is 0.2 μm^2), their partial volume (portion of cell cross section that is occupied by organelles, v/v) is 4.7%; in RCC they are rare (1.3), large (3.4 μm^2), v/v is 1.7%. In RCC leucoplasts are large (1.3 μm^2) but few (4.2), v/v is 20%, they have contacts with ER at about 7% of their surface. They contain solitary thylacoids and few plastoglobuli. In RCC leucoplasts are small (0.5 μm^2), few (2.3), v/v is 7%, but they have contacts with ER at about 32% of their surface. They contain dilated thylacoids filled with opaque substance and protein bodies surrounded by plastoglobuli. Ultrastructural data shows that the canals may differ by their exudation composition. The study was supported by RSCI (N 04-04-49417).

P1958. Ultrastructure of terpenoid-secreting trichomes on leaves of *Plectranthus ornatus* (Lamiaceae)

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In a previous work we characterized five morphological types of secretory trichomes on leaves and flowers of *Plectranthus ornatus*. Here we present the ultrastructure of peltate and long-stalked capitate trichomes, where the bulk of essential oils from the leaves is produced. In both trichomes, at onset of secretion, the lateral stalk walls undergo cutinisation. During the active secretory phase, the most striking ultrastructural features of glandular cells are the hypertrophy of leucoplastidome and the extensive proliferation of tubular SER. The high development of these two cell compartments is related to the biosynthesis and transport of terpenoids. A granulocrine secretion mechanism may operate alone or concurrently with an eccrine process. Secretory products accumulate in a large subcuticular space. Whereas in peltate

trichomes the secretion (an oleoresin) remains trapped in the subcuticular space, in long-stalked capitate trichomes the secretory products (a gumresin) are released by cuticular rupture. During the postsecretory phase a progressive degeneration of the glandular cells is observed. Secretion may be considered mero-holococrine.

P1959. Cytological development, metabolite secretion and molecular identification of terpenoid cyclases in sunflower glandular trichomes

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Secretion of sesquiterpene lactones (STL) in capitate glandular trichomes from the anther appendages of *Helianthus annuus* was observed during development of the florets within the sunflower capitulum. All stages of forming the cuticular globe, from the presecretory to the postsecretory phase, were studied microscopically. The secretory activity was simultaneously monitored using 6 STL for quantitative HPLC analysis. Thereby, beginning and termination of the STL biosynthesis was defined in relation to floret ontogenesis, particularly the pollen formation. Part of the secreted material showed autofluorescence due to the presence of a 5-deoxygenated hydroxy-trimethoxy-flavone.

cDNA from anther trichomes in their biosynthetic active stage was used for homology-based PCR. This resulted in 2 DNA fragments of putative germacrene synthases with approximately 550bp and 600bp in length and nucleotide identity of 83% and 67%, respectively, to corresponding germacrene synthases from *Lactuca sativa*.

P1960. *Ent*-Kaurene Diterpenes from *Rabdosia excisa* and Their Chemical Conversion

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Rabdosia excisa Hara (Labiateae), which has been used as a folk medicine for treatment of fever and arthralgia in north China. A number of diterpenes from *Rabdosia* plants are *ent*-kaurene diterpenes having cytotoxic, antibacterial, NF- κ B activation-inhibiting, and apoptosis-inducing activities. In our present chemical investigation of this plant, collected in Jing Yu County, Jinlin Province of China, we isolated four novel *ent*-kaurene diterpenoids and eight known ones. Furthermore, facile transformation of 7,14-dihydroxy-*ent*-kaurenes such as excisanin A, kamebanin, and kamebakaurin, which are abundant in the plants, to *ent*-abietanes was accomplished under the Mitsunobu reaction conditions. The α , β -unsaturated *cis*-fused β -methylene γ -lactones thus prepared and the isolated diterpenes were applied to cytotoxic activity on P388 murine leukemia cells. In this congress, we report the isolation, structural elucidation, chemical conversion, and cytotoxic activity of these *ent*-kaurenoids.

P1961. Cloning and expression of a farnesyl diphosphate synthase cDNA from *Centella asiatica* (L.) Urban

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A cDNA encoding farnesyl diphosphate synthase (FPS; EC2.5.1.1/EC2.5.1.10) was isolated from *Centella asiatica* (L.) Urban, using degenerate primers designed from two highly conserved domains. A full-length cDNA clone was subsequently isolated by rapid amplification of cDNA ends PCR. The sequence of *CaFPS* (*C. asiatica* farnesyl diphosphate synthase) contains an open reading frame of 1029 nucleotides which codes for 343 amino acids with a molecular mass of 39.6 kDa. The deduced amino acid sequence of *CaFPS* exhibits 84%, 79%, and 72% identity to those of the *Artemisia annua*, *Arabidopsis thaliana*, and *Oryza sativa* farnesyl diphosphate synthase, respectively. Southern blot analysis showed that the *C. asiatica* genome might contain only one gene for FPS. The artificially expressed soluble form of the *CaFPS* enzyme was identified by SDS-PAGE and *CaFPS* exhibits high specific activity that produces farnesyl diphosphate as the major isoprenoid.

P1962. 3beta-Hydroxysteroid dehydrogenase (3beta-HSD) from *Erysimum crepidifolium* Rchb.

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In order to extend our knowledge on the evolution of the cardenolide trait in angiosperms we studied cardenolide biosynthesis in the genus *Erysimum* (Brassicaceae). *E. crepidifolium* Rchb. and *E. allionii* hort., used in this study, accumulate strophantidin-type cardenolides to considerable levels. We focussed on two of the early biosynthetic steps, namely the conversion of pregnenolone to progesterone and the conversion of progesterone to 5-pregnane-3,20-dione, catalyzed by 3-hydroxysteroid dehydrogenase (3-HSD) and 5-progesterone reductase (5-POR), respectively. Several starting materials, such as leaves, callus and cell-suspension cultures were checked and we found *E. crepidifolium* and its *in vitro* cultures to be more suitable than *E. allionii*. The activities of the two target enzymes was detected by TLC and HPLC and the conditions for isolating and characterizing 3-HSD were optimized. Since oligonucleotide primers generated on the basis of the 3-HSD cDNA isolated from *Digitalis lanata* were not suitable to isolate the gene directly, the protein is to be purified and sequenced.

P1963. Cloning of a cDNA encoding putative -amyrin synthase associated with asiaticoside biosynthesis from *Centella asiatica* (L.) Urban

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A homology-based PCR method was used to clone a cDNA for oxidosqualene cyclase from *Centella asiatica*, which produces a large quantity of triterpene saponins such as asiaticoside and madecassoside. Sequence analysis of one clone found sequences related to beta-amyrin synthase. ORFs of the full-length clones were named CabAS (*Centella asiatica* putative beta-amyrin synthase). On the basis of amino acid sequences, CabAS appears to be an enzyme (beta-amyrin synthase), which synthesizes beta-amyrin. Southern analysis showed that the *C. asiatica* genome contains one copy of the putative beta-amyrin synthase gene. Northern blot analysis demonstrated that the CabAS gene is expressed in leaves with no detectable transcript in other plant tissues, consistent with the organ-specific accumulation of the asiaticoside. Also, up-regulation of expression of CabAS by methyl jasmonate in leaves was demonstrated. This suggests that beta-amyrin synthase may play an important role in enhancement of saponin production, including asiaticoside.

P1964. Genetic Transformation of *Ajuga multiflora* Bunge with *Agrobacterium rhizogenes* and 20-Hydroxyecdysone Production in Hairy Roots

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An efficient transformation system for *Ajuga multiflora* Bunge was established by using *Agrobacterium rhizogenes* A4 strain. A number of hairy-root clones were obtained from micro-calli of leaf petioles and leaf explants after inoculation with the bacteria. One of the various lines was revealed to be a fast-growing line. This line also showed the highest production of 20-hydroxyecdysone (20-HE). The polymerase chain reaction amplification of rooting locus genes showed that left-hand transferred DNA of the root inducing plasmid of *A. rhizogenes* was inserted into the genome of transformed *A. multiflora* hairy roots. Integration of rooting locus genes into genome of transgenic plants was further confirmed by Southern hybridization. The content of 20-HE in hairy root was 10 times higher than that in wild-type roots. These results indicate that hairy roots of *A. multiflora* are able to produce higher 20-HE than wild-type roots and considered as the high efficient culture system for 20-HE production.

P1965. Studies on *Thymus teucrioides* subsp. *candilicus*: Chemical composition of the essential oil content of the glandular trichomes, using a novel isolation method.

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Thymus teucrioides Boiss. & Spruner subsp. *candilicus* (Beauverd) Hartvig (Lamiaceae) is a subspecies of the genus *Thymus* growing wild in Greece. Micromorphological studies in *Thymus* showed that this taxon is characterized by the presence of abundant glandular trichomes on the vegetative and reproductive organs surface secreting mixtures of secondary metabolites of biotechnological and pharmacological interest in high quantities. We tested a novel method to obtain the content of glandular hairs directly, using a micropipette. The mixture was further analyzed using GC/MS and found to be rich in p-cymene (46.1-58.3%), p-cymene-2,3-diol (10.4-12.2%) and 3-hydroxy-2methyl-5isopropyl-p-benzoquinone (7.2-12.1%). The proposed method gives a different composition of the essential oil compared to the Micro-Steam Distillation Extraction Method (MSDE). The high essential oil's yield was determined using the Hydrodistillation method. Micromorphological and chemical results showed that the proposed isolation procedure might become a useful tool to obtain the content of glandular trichomes without significant changes on their *in vivo* composition.

P1966. cDNA isolation and functional expression of Linalool Synthase from *Nicotiana alata*

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Flowers of *Nicotiana alata* (Solanaceae) emit a broad range of volatile organic compounds (VOCs), comprising primarily mono- and sesquiterpenes and aromatic esters. Linalool, an acyclic monoterpene, is a major component emitted from *N. alata* flowers during the night. A cDNA encoding a monoterpene synthase was isolated via RT-PCR from petals. The nucleotide sequence of this terpene synthase consists of 1629 bp encoding a protein of 542 amino acids. The protein sequence is similar to other monoterpene synthases from different plant families. The heterologous expression of the tps clone allowed purification and characterization of this enzyme. It converts geranyl diphosphat into linalool and is therefore designated Linalool Synthase (LIS), although the protein shows highest identity at the amino acid level to an -terpineol synthase from *Vitis vinifera*. The *lis* gene is only expressed in petals and anthers, not in other flower and plant tissues. High transcript levels during night correlate with the nocturnal emission.

P1967. Unraveling the wizardry of terpene biosynthetic enzymes

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Many plants respond to pathogen attack by the synthesis and secretion of anti-microbial compounds. For example solanaceous plants produce anti-microbial terpenes that inhibit germination and growth of several fungal species. The production of these chemicals has therefore been interpreted as an important defense response. We have also hypothesized that an understanding of the mechanisms responsible for the biosynthesis of the anti-microbial terpenes should provide a means for engineering the generation of novel and more efficacious compounds. Towards that goal, we have elucidated a 2-step biosynthetic pathway for capsidiol, an anti-microbial sesquiterpene di-alcohol. The pathway consists of a terpene synthase that catalyzes the cyclization of farnesyl diphosphate to a bi-cyclic hydrocarbon structure, followed by the action of a P450 hydroxylase that introduces 2 hydroxyl functions with stereo- and regio- specificity. Using a combination of methods, we have mapped functional features of the respective enzymes, and more recently have used this information to evolve

novel catalytic activities for the generation of unique chemical entities.

P1968. Phenolic constituents from *Juniperus* plants

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In our study of the chemical constituents in *Juniperus* plants, we investigated the structures and biological activities of phenolic components of *Juniperus communis* L. var. *depressa* Pursh and *J. occidentalis* Hook. collected in Oregon, U.S.A. The genus *Juniperus* belongs to family Cupressaceae and comprises about seventy species distributed in the Northern Hemisphere. From the methanolic extracts of the two *Juniperus* plants, 22 flavonoids, 15 neolignan (lignan) glycosides and 17 phenylpropanoids (phenylethanoids) were isolated and their structures were established on the basis of spectral and chemical evidence. Among them, 14 isolates were new compounds. The inhibitory activities of some isolated compounds on the Maillard reaction and lipid peroxide formation were examined. Some flavonoids showed inhibitory activities. In addition, thin-layer chromatograms of some *Juniperus* species distributed in Japan are reported for comparisons in chemical components. Flavonoid components are also discussed from chemotaxonomical viewpoints.

P1969. Studies on color change of blue morning glory and pH increase during flower opening

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Petals color of morning glory, *Ipomoea tricolor* cv. Heavenly blue, changes from reddish-purple to blue during flower opening. The color change is caused by an unusual vacuolar pH increase from 6.6 to 7.7 of the adaxial and abaxial colored cells. To clarify the alkalization of epidermal vacuoles we focused on vacuolar membrane proteins. We isolated colored protoplasts from buds and full-opened petals and purified vacuolar membranes. The membranes contained V-ATPase, V-PPase and NHX1, which were immunochemically detected, with relatively high transport activity. NHX1 could be detected only in the vacuolar membranes of full-opened petals. These results suggest that the vacuolar pH increase of morning glory petals is due to an active transport of Na⁺ and/or K⁺ from cytosol into vacuoles through a sodium driven NHX1, which is supported by V-PPase and V-ATPase. This systematic ion transport brought alkalization of vacuolar pH to resulted sky-blue flower petals.

P1970. Studies on blue sepal color development of *Hydrangea macrophylla*

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The sepal color of *Hydrangea macrophylla* is very famous for ready change of hues. All colored sepals, red through purple to blue, contain the same anthocyanin, delphinidin 3-glucoside and the same co-pigments, quinic acid derivatives. It is well known that acidity of the soil influences sepal color; the higher the Al³⁺ content of hydrangea growing in the acidic soil is, the bluer the sepals become. Thus, there must be some interesting system for developing the wide variety of colors. Furthermore, the structure of blue color pigment is still obscure. To clarify the blue color development of hydrangea we reconstructed blue solution by mixing the sepal components. When Al³⁺ was added to delphinidin 3-glucoside in buffered solution blue-black colored mass was quickly precipitated indicating that aluminum complex of the anthocyanin is insoluble in an aqueous solution. However, the mixture of the anthocyanin, Al³⁺, and 5-O-caffeoylquinic acid gave a stable blue colored solution the same color as hydrangea sepals. Therefore, the quinic acid derivative have a strong co-pigment effect on Al-complex of the anthocyanin.

P1971. Pinoresinol-Lariciresinol Reductases from Justicidin B producing cell cultures of *Linum perenne*

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Lignans are dimers of phenylpropanoid units. For the first time we have isolated the aryl-naphthalene lignans justicidin B and isojusticidin B and glycosides of diphyllin from suspension cultures of *Linum perenne* Himmelszelt. Pinoresinol-lariciresinol reductase (PLR) is discussed as an early general step in the biosynthesis of lignans [Davin and Lewis (2003) Phytochem. Rev. 2, 257-288]. Therefore, to get first insights in the biosynthetic pathway of aryl-naphthalene lignans we isolated a partial cDNA sequence probably encoding PLR from *L. perenne* Himmelszelt. We will isolate a full length clone and express the protein in *E. coli* to prove its functionality. To show whether the PLR is involved in the biosynthesis of aryl-naphthalene lignans in *L. perenne* we will transform a RNAi construct into *L. perenne* Himmelszelt with the help of *Agrobacterium rhizogenes*. We could already set up a transformation protocol for this plant species. The resulting hairy roots contain the same lignans like the suspension cultures.

P1972. Phenylpropanoid- and apigenin-glucoside derivatives in the course of development of *Matricaria chamomilla* ligulate flowers

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Matricaria chamomilla synthesizes (Z)- and (E)-2'-D-glucopyranosyloxy-4-methoxy cinnamic acids as one of the main phenolic secondary metabolites of shoot and flower. Apigenin-7-O-glucoside and its acylated derivatives are accumulated exclusively in ligulate flowers. Quantity of the compounds was estimated in six phases of development of ligulate flowers of diploid and tetraploid chamomile cultivars. (E)-2'-D-glucopyranosyloxy-4-methoxy cinnamic acid, the precursor of phytoanticipin herniarin, is accumulated in higher concentrations in young growing ligulate flower, during flowering and post-flowering phases its content decreases. Tetraploid plants of chamomile with higher content of defence compounds probably raise power of resistance in comparison with diploid plants. The content of apigenin glucoside derivatives in ligulate flowers of diploid plants was found significantly higher before start of flowering. The study was supported by the Grant Agency VEGA (no. 1/0444/03).

P1973. Structure analyses of polyacylated anthocyanins and other polyphenols in the leaves of *Gynura bicolor* DC. and estimations of their radical scavenging activities

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Gynura bicolor belonging Compositae is vegetables cultivated in Southeast Asia and Japan. The under surface of the leaves is colored in purple by anthocyanins. We isolated the pigments and analyzed their structures by UV, NMR and Mass spectra. One of the two major anthocyanins was identified as 3-O-(6-O-malonyl-D-glucopyranosyl)-7-O-(6-O-(4-O-(6-O-caffeoyl-D-glucopyranosyl)caffeoyl)-D-glucopyranosyl)-3'-O-(6-O-caffeoyl-D-glucopyranosyl)cyanidin, which was identical with the polyacylanthocyanin isolated previously by us from purple petals of *Senecio cruentus* belonging Compositae and named as rubrocinerarin. The other anthocyanin was characterized as malonylrubrocinerarin, the analysis of the bond position of a malonyl residue being in progress now. The vegetables contained some flavonoids and chlorogenic acid in addition to anthocyanins, the flavonoids being identified as 3-O-glucoside and 3-O-galactoside of quercetin / kaempferol. Rubrocinerarin and the other flavonoids showed high scavenging activities of DPPH radical.

P1974. Color variations and flavonoids in the flowers of *Clematis patens* (Ranunculaceae)

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The flower colors of *Clematis patens*, which is listed in "Threatened Wildlife of Japan", varied from white, pink to purple. In this survey, the anthocyanin and other flavonoid composition in various flower colors of the species was compared by HPLC characterized. Major anthocyanin of purple flowers was characterized as delphinidin caffeoylglucoside with minor cyanidin glycoside. Though both the anthocyanins were contained in pink flowers, they were small in quantity as compared with those of purple flowers. In white flowers, some flavonols but not anthocyanins were present and seven were estimated as kaempferol 3-caffeoylglucoside, 3-glucoside, 3-rutinoside, 3-gentiobioside, 3-alloside and two 3-glycoside. In pink flowers, the former two glycosides were detected as major compounds and other ones as minor. However, their flavonols were apparently absent in purple flowers on HPLC chromatogram.

P1975. Flavonoid diversity of *Saussurea* species in the Tien Sian Mountains

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The genus *Saussurea* (Compositae) is widely distributed from alpine region to low land of eastern Asia, and is morphologically varied. In this survey, the flavonoids were isolated from seven *Saussurea* species, *S. sordida*, *S. schanginiana*, *S. elegans*, *S. involucreta*, *S. leucophylla*, *S. gnaphalodes* and *S. setaraphifolia*, in the Tien Sian Mountains of central Asia. They were identified as quercetin 5-glucoside, quercetin 7-glucoside, isorhamnetin 5-glucoside, kaempferol 5-glucoside, luteolin 7-glucoside from *S. sordida*, isoorientin, isovitexin, quercetin 3-rutinoside from *S. schanginiana*, velutin, luteolin 7-methyl ether from *S. leucophylla*, luteolin 7-glucuronide, selagin, selagin 7-methyl ether from *S. gnaphalodes*, nepetin, hispidulin, hispidulin 7-glucoside from *S. involucreta* and so on, by UV, NMR, MS manners. From the results described above, the flavonoids of *Saussurea* species are complex, and chemical diversification of the genus is chemotaxonomically interested.

P1976. Peroxynitrite attacks prenylhydroquinone glucoside: A NMR analysis.

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Some plant phenolics are recognised as scavengers of peroxynitrite (ONOO⁻), an anion formed as a result of the combination of nitrogen monoxide and superoxide (O₂⁻). At neutral or acidic pH, ONOO⁻ is converted into peroxynitrous acid, which decomposes to nitrogen dioxide, a free radical able to nitrate aromatic aminoacid residues. The mechanism underlying the effect of phenols is their susceptibility to undergo either oxidation to benzoquinone or nitration of the activated aromatic ring [1].

In the present study we report on the chemical modifications involved in the reaction of ONOO⁻ and 2-dimethylallylhydroquinone-1-glucoside (DHG), one of the active principles of *Phagnalon rupestre* (Asteraceae). Examination of the ¹H- and ¹³C-nuclear magnetic resonance spectra of the products formed indicated that DHG undergoes mono- and dinitration, depending on the molar ratio of the reagents. High ONOO⁻ concentration causes degradation.

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References: 1. Pannala A. *et al.* (1998) Free Radic. Biol. Med. 24: 594-606.

P1977. Comparison of anthocyanin and flavone pigments between blue and other flower colors of *Centaurea cyanus*

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It has been reported that the blue flower of *Centaurea cyanus* is due to coexistence of an anthocyanin, cyanidin 3-O-(6-succinylglucoside)-5-O-glucoside and a flavone, apigenin 7-O-glucuronide-4-O-(6-malonylglucoside). However, the pigments of other flower colors are not reported, except pink flower. In this survey, the pigments were isolated from various flower color cultivars and identified, and compared with that of the blue flower. White, pink, red, reddish purple and purple flowers of *C. cyanus*

were used as materials. Purple flower of *C. cyanus* contained the same flavone with blue, but major anthocyanin was different. In reddish purple, though the anthocyanin was the same with blue, flavone was different. In pink and red flowers, anthocyanin was pelargonidin. Moreover, the flavone of latter was also different from blue. Consequently, it was presumed that various flower colors of *C. cyanus* are revealed by the combination of quantitatively different anthocyanins and flavones.

P1978. Aloe arborescens. Plant chemistry and chemical defense systems.

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Aloe arborescens Miller (Liliaceae) is a dense, shrubby plant. It is widely used as a hedge to protect agricultural fields. It is possible the most widely cultivated aloe in the world and can be seen grown in gardens in many cities around the world.

Aloe arborescens has attracted public attention as a plant which can be used as a natural health food and also in cosmetics and medicinal drug applications.

According to some studies *A. arborescens* is richer than *A. Vera* in respect to medicinal properties.

During the last 14 years screening of *A. arborescens* and other *A.* species have been carried out. The secondary phenolic metabolites (SPhMs), bioactive compounds, such as barbaloin, aloeresin and aloenin have been found in the *A. arborescens* leaves. The distribution of the SPhMs in *Aloe arborescens* leaves depends on the leaf part, age, position of the leaf, after pruning leaves, as well as on seasonal influences. Perhaps most are involved in chemical defense systems, which protect plants from herbivory and from other destructions.

P1979. Formation of UV-honey guides in *Rudbeckia hirta*

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Honey guides are part of the flower pigmentation pattern, which serves to attract the pollinator to the sex organs and nectar of the particular blossom. In *Rudbeckia hirta*, honey guides are formed by the accumulation of higher hydroxylated, UV-absorbing flavonol glycosides, which are invisible to the human eye but detectable by insects. In this studies, biochemical investigations were performed on the flavonoid enzymes, which are involved in the formation of the higher hydroxylated flavonol glycosides in *Rudbeckia hirta*: flavonol-6-hydroxylase, flavonol-7-O-glucosyltransferase and flavonol-6-methyltransferase. Enzymatic formation of quercetagenin-7-O-glucoside, 6-methoxyquercetin-7-O-glucoside and 6,7-dimethoxy-quercetin-3-O-glucoside was demonstrated for the first time in *Rudbeckia hirta* (cultivars 'Goldilocks' and 'Indian Summer'). In addition, the distribution pattern of this flavonols were investigated spectrometrically.

P1980. Anthocyanins in the flowers of *Gladiolus* cultivars

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The *Gladiolus* is largest genus in the family Iridaceae and consists of ca. 250 species. In addition, many cultivars were made by crossing among numerous species. So far, some anthocyanins have been identified as flower pigments from some cultivars.

In this survey, the anthocyanins of six cultivars, *G. × grandiflora* 'Ariake', 'Beijing', 'Jyuku-gaki', 'Fado', 'Ben Venuto', 'Marches' and 'Vioretta' were analysed. Eleven anthocyanins were isolated from the flowers and identified as 3,5-di-O-glucosides of pelargonidin, cyanidin, peonidin and malvidin, two pelargonidin and malvidin glycosides, and each one cyanidin, peonidin and petunidin glycosides. The anthocyanin composition of 89 cultivars, which are representative red, purple, reddish purple and pink, were surveyed by HPLC. The distribution patterns of anthocyanins were divided into some groups, i.e., red, purple, reddish purple and pink into 3, 2, 2 and 5 groups, respectively.

P1981. Red Leaf Flushing in Tropical Plants

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Red-leaf flushing is a conspicuous phenomenon in tropical woody plants due to the production of anthocyanins in developing leaves and shoots. It has been suggested that the red colouration can provide protection against photodamage or by making the leaves cryptic to herbivores. This project investigated red-flushing by comparing the herbivory rate of 12 red-flushing species with 8 normal-greening species. Red young leaves were grazed more rapidly than both normal greening young leaves and mature leaves of red-flushing species. Red young leaves showed no increased resistance, or were even more susceptible, to herbivory than controls. Measurement of the rate of photosynthesis revealed that young red leaves showed smaller inhibition at high light intensity when compared to green young leaves. For dark recovery following photoinhibition, anthocyanin-pigmented young leaves were able to recover 69% of fluorescence yield while non-anthocyanin-pigmented young leaves only recovered 58%. Red young leaves were more resistant to photo damage supporting the photoprotection hypothesis.

P1982. On the relationships between isoprene emission and light and dark respiration in hybrid poplars under free-air CO₂ enrichment

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Climate change may have contrasting effects on isoprene emitted by forest. While elevated CO₂ may reduce isoprene emission, rising temperature is expected to increase this rate. Rosenstiel *et al.* (2003) data suggested that isoprene emission competes with mitochondrial respiration for cytosolic phospho-enolpyruvate. Increasing rates of mitochondrial respiration driven by rising CO₂ and temperature may therefore be responsible for the decrease of isoprene emission observed in laboratory and field experiments under high CO₂. We tested this in a *Populus x euramericana* plantation grown in free air CO₂ enrichment (FACE). Isoprene emission and light and dark respiration were measured on-line in developing and developed leaves at the temperatures of 25 and 35 °C. For isoprene emissions lower than 30 nmol m⁻²s⁻¹, an inverse relationship was observed with light and dark respiration, in both CO₂ treatments. However, when isoprene emissions are enhanced, principally by exposure to elevated temperature, this relationship is reversed, and light and dark respiration increase concurrently with isoprene emission.

Supported by ESF-VOCBAS scientific programme.

P1983. Changing the none-isoprene emitting model plant *Arabidopsis thaliana* into an isoprene emitter by genetic transformation

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There has been growing research focusing on the volatile hemiterpene isoprene. It is known that volatile isoprenoids emitted by vegetation have various meanings, i.e. in plant defence, but also in atmospheric chemistry. However, the physiological function of isoprene for plants itself is still unknown. Actual studies indicate that isoprene can enhance thermotolerance or quench oxidative stress; however the underlying mechanisms are widely unknown. Aim of the presented work is to study the proposed biological function of isoprene formation in transgenic *Arabidopsis thaliana*. Plants have been transformed with different constructs of an isoprene synthase gene (*ispS*) from grey poplar as well as with the deoxyxylulose 5-phosphate reductoisomerase (*dxr*) gene from poplar catalysing one possible committed step of the MEP-pathway in order to enhance the metabolite flux within the pathway. The presented data show our selection of transgenic lines and their ability to produce and emit isoprene.

P1984. Chemical and Morphological Investigations And Habitat Specificity of Endemic *Salvia plifera* Montbret & Aucher ex Benth from Turkey

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The genus *Salvia* comprises 89 species which are found in Turkey and ratio of endemism in the genus is % 50. *Salvia plifera* is known in Central, South and West Anatolia (300 between 1320 m altitude). *S. plifera* plants were collected from South East Mediterranean Region of Turkey Kahramanmaraş Ahirdag provinces at different altitudes among bushes and macchies in

June. Morphological characteristics were determined both macroscopically and stereomicroscopically. Some morphological differences were established according to altitudes and Flora of Turkey.

The air dried flowering parts of the plants were hydrodistilled with clevenger type apparatus and the essential oil content was found as 0.1%. The chemical composition of the essential oils was determined with GC/MS and nineteen components identified in the oil representing 96.4% of the total oil. *S. plifera* oil had high levels of 1.8 cineol (12.4 %), -pinene (10.2 %) and -pinene (10 %), -thujone (8.7%) and -caryophyllene (8%).

P1985. Isoprene interaction with nitric oxide and reactive oxygen species in leaves exposed to high ozone.

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Isoprene reduces ozone damage to leaves by an unknown mechanism. Fumigation with high doses of ozone stimulated isoprene emission from *Phragmites* leaves, bringing further, indirect, evidence of its antioxidant role. The photosynthetic apparatus of leaves in which isoprene emission was inhibited by fosmidomycin was more damaged by ozone than in isoprene-emitting leaves. Three days after ozone fumigation, the necrotic leaf area was higher in isoprene-inhibited leaves than in isoprene-emitting leaves. Isoprene-inhibited leaves exposed to ozone accumulated antioxidant enzymes, reactive oxygen species (ROS, namely H₂O₂), products of lipid peroxidation, and nitric oxide (NO). It is speculated that isoprene may react with peroxy nitrates formed by NO in presence of ROS, draining NO. In nature, the simultaneous quenching of NO and ROS by isoprene may be an effective mechanism to control dangerous compounds formed under stress conditions, while attenuating the induction of hypersensitive responses leading to cellular damage. Supported by ESF-VOCBAS and EC-MCRTN (ISONET).

P1986. The role of exogenous isoprene in protecting *Arabidopsis* mutants deficient in the xanthophylls cycle (NPQ1) from high light

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Isoprene was proven to have a thermo-protective role and to decrease ozone damage. In order to expand our understanding of isoprene functions in leaves, we investigated whether exogenous isoprene could replace the photoprotective role of other isoprenoids, namely xanthophylls. Leaves of transgenic *Arabidopsis* NPQ1 plants, defective in the xanthophylls cycle, being unable to convert violaxanthin to zeaxanthin in the light, were used. NPQ1 mutants were compared to wild types (cv. Columbia) for photosynthesis, fluorescence properties, electron transport rate, antioxidant status and reactive oxygen species (ROS) production, when exposed to excessive light in presence or absence of exogenous isoprene. Preliminary results indicate that, when exposed to exogenous isoprene, NPQ1 leaves became less sensitive to photoinhibition and produced less ROS. This suggests that isoprene might in fact alleviate photodamage although it remains to be studied whether this effect be due to a biochemical mechanism related to the xanthophylls cycle. (Supported by MCRTN-CT-2003-504720, ISONET)

P1987. Development of a transient expression system in poplar protoplasts: A tool for the study of volatile terpenoids physiological role.

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Many plant species emit volatile terpenoids whose functions are diverse and not always well characterized. To understand their physiological role and to better predict their release into the atmosphere where they are highly reactive, studies are conducted on different species, including poplars which are strong emitters of isoprene. It has been shown that in poplars isoprene emission and its synthesizing enzyme (isoprene synthase) gene expression follow seasonal and diurnal variations. Aim of the presented work was to develop a transient expression assay system with mesophyll poplar protoplasts (*Populus x canescens*). The

presented work shows that these protoplasts emit isoprene after incubation under light. To better characterize the regulatory factors of isoprene emission and its possible physiological function at the cellular level, constructs aimed at over-expressing or repressing isoprene synthase as well as other terpene synthase genes will be introduced into these protoplasts.

P1988. Monitoring terpene emissions by high elevation terrestrial ecosystems in response to global changes - REMOTE

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The impact of anthropogenic pollution and global change on terrestrial ecosystems is investigated worldwide. Remote ecosystems at high altitudes are exposed to background levels of on-site anthropogenic pollutants and greenhouse gases, but may be subjected to regional and global trends of air composition changes, and to transboundary pollution. The REMOTE project will assess impacts of increased pollution and climate changes on forest ecosystems of high altitudes in the Himalayan, Alpine and Andine regions. Supported by the Istituto Nazionale Ricerca sulla Montagna (INRM), REMOTE will focus on ecosystem functional and structural features. A large effort is planned to screen forest emissions of Volatile Organic Compounds (VOC), studying the relations between VOC and assimilated carbon, and focussing on VOC (terpenes) of importance in atmospheric chemistry. An environmental and ecophysiological database will be created. The project outputs will be useful to parameterize the impact of the environment and long-range pollution on remote forest of mountain areas, and to develop models predicting the role of these ecosystems in atmospheric chemistry.

P1989. Reasons of chemotypic variation in *Thymus pulegioides* L.

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The influence of change of environmental conditions on chemical composition of essential oils in five different chemotypes of *Thymus pulegioides* L. was examined. The plants collected in various natural habitats were planted into a cultivated field under even soil conditions and propagated clonally every year for 5 years. The essential oil composition was monitored by capillary GC and GC/MS. It was found that according to the stability of essential oil composition after the change of environmental conditions two groups of *T. pulegioides* can be distinguished: 1) plants which preserve their chemical composition of the essential oils; 2) plants which considerably change their chemical composition of the essential oils. This may be explained by the dependence of the interaction of genes on the environmental factors. Some alleles, which determine the chemotype of plants, may condition a wider reaction range or their interaction with certain genes depends more on the environmental factors.

P1990. Effect of high phosphorous on isoprene emission from *Phragmites australis* in reconstructed wetlands.

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Phragmites australis is a strong isoprene emitter. Isoprene can contribute to air pollution when reacting with anthropogenic compounds but *Phragmites* is used to phytoremediate polluted wastewater in urban environments. Isoprene emission requires phosphorylated compounds and may be controlled by inorganic phosphorous level (Pi). We are determining whether excess of Pi, normally occurring in reconstructed wetlands used for phytoremediation, can regulate isoprene emission, possibly increasing its load in the urban atmosphere. Three groups of plants are growing under three different levels of KH₂PO₄ and controlling all other environmental variables. Preliminary results indicate that basal levels of isoprene emission from *Phragmites* are less sensitive than photosynthesis to Pi and are not stimulated by high Pi. Plants growing in reconstructed wetlands may remove

high levels of Pi from water without increasing the load of isoprene in the atmosphere. *Research supported by ESF-VOCBAS.*

P1991. Stress induced emission of biogenic VOC: Induced sesquiterpene emissions from ozone exposed tobacco

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The kinetics of stress induced VOC emissions were studied after ozone (O₃) exposures of tobacco Bel W3. After O₃ exposures we observed increased emissions of methanol, LOX products, methyl salicylate (MeSA), and sesquiterpenes (SQT) starting with lag times of 0.5 - 16 h. These lag times were dependent on O₃ uptake and VOC species. Methanol emissions increased first, followed by those of MeSA and LOX products.

SQT emissions increased in some cases before in other cases simultaneously to those of LOX products and MeSA. SQT emissions often showed two peaks and their emission pattern was not constant implying at least two different plant internal signals leading to increased SQT emissions. Exposures with methyl jasmonate or with MeSA led to increased SQT emissions but with patterns significantly differing from those observed after O₃ exposures. Exposing the plants to ethene led to increased SQT emissions with a pattern very similar to that during the first peaking SQT emissions after O₃ exposure. Hence, we believe that in case of ozone exposed tobacco neither accumulation of jasmonic acid nor of salicylic acid is responsible for increased SQT emissions.

P1992. Terpene content and headspace of in vitro cultures of *Myrtus communis* L.

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Micropropagation of myrtle has been reported for genotypes of various sources and could allow rapid clonal propagation, germplasm conservation and is a basic prerequisite for the exploitation of biotechnological techniques. Terpene headspace and tissue content of in vitro cultures of different clones of myrtle characterized by different morphological features and geographic origin have been investigated. Headspace technique combined with adsorption on solid materials and to GC-MS analysis was applied for the determination of volatiles emitted by plants in vitro cultured. The terpene content was evaluated after extraction with solvent and analysis by GC-MS. Several terpenes were detected in both headspace and tissue content of *Myrtus communis* cultures. The main terpene components of in vitro plant headspace were isoprene, α-pinene, p-cymene and other compounds in smaller quantities such as 1,8-cineole. The main terpene components of microcutting content were α-pinene, linalool, limonene and 1,8-cineole. Each clone was characterized by a unique profile of headspace and tissue content of terpene. A limonene chemotype was detected.

P1993. BVOCs produced by *Pyrus communis* in response to fire blight infection

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Few studies have been carried out on volatiles specifically aging between the bacteria and plants. Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill), is the most destructive bacterial diseases of pomaceous plants such as apple (*Malus domestica*) and pear (*Pyrus communis*). Fire blight is characterized by a long asymptomatic phase, therefore an effective diagnosis is very important to avoid further spread of the disease. The present contribution reports preliminary results on the possible role of volatiles produced during the interaction *Erwinia amylovora*/pear plants using the bag-enclosure system combined with adsorption on solid materials and to GC-MS analysis and the electronic nose. Specific volatiles were detected in the plant/bacterium system, while electronic nose was able to distinguish between the infected plants and the control ones, showing the possibility to perform an early diagnosis of the disease in asymptomatic plant via the use of this system.

P1994. Isoprenoid emissions from three *Nothofagus* species in Patagonia Argentina

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Earlier studies, concerning the occurrence of isoprenoid production within the plant kingdom, showed that none of the measured species of the Fagaceae family (i.e. *Fagus*, *Nothofagus*, *Chrysopsis*, *Castanea*, and *Lithocarpus*) exhibited isoprene emission. However, a recent study showed that *Fagus sylvatica* (European beech) was an emitter of monoterpenes. The *Nothofagus* (southern beech) species is a woody plant native of the southern Hemisphere. Three *Nothofagus* (*N. antarctica*, *N. pumilio*, and *N. dombeyi*) species, growing in natural forests in Bariloche (Argentina), were screened for emissions of biogenic volatile organic compounds (BVOCs). *N. antarctica* and *N. pumilio* are deciduous species, whereas *N. dombeyi* is an evergreen species. Basal emissions of isoprenoids measured at photosynthetic photon flux density of 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and at a leaf temperature of 30 °C in fully expanded leaves of adult trees, indicated that all the tested *Nothofagus* were monoterpene emitters but at different emission rates.

P1995. Changes in the cuticular waxes during tomato fruit development and their importance as a transpiration barrier

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During ripening of tomato (*Lycopersicon esculentum* MILL.) fruits, cuticular waxes undergo significant changes. From the onset of fruit development (immature green fruits) up to mature red fruits total soluble wax load increases by a factor of 3.5. This quantitative effect of surface waxes changes in a characteristic way, involving compositional alterations in cyclic as well as aliphatic components. Along with these chemical alterations the cuticular transpiration decreases dramatically.

The aim of the present project is to elucidate the direct correlation between the cuticular wax composition and its transpiration barrier properties as it is possible due to the astomatous surface of intact tomato fruits.

In addition, a tomato -ketoacyl-CoA synthase deficient wax mutant (*lecer6*) is analysed in these two parameters during fruit ripening. The comparison of mutant and wild-type fruits leads to a more detailed understanding on the importance of the cuticular waxes, especially of their chemical composition, on cuticular transpiration. The developmental changes in the composition of cuticular wax are discussed with regard to their biosynthesis.

P1996. Relevance of cuticular wax properties for the interspecific interaction of powdery mildew with its host plant barley

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As an obligate biotrophic fungi *Erysiphe graminis* depends on *Hordeum vulgare* host cells to obtain products of plant metabolism for nutrition and reproduction. The plant cuticular waxes are the first contact site and are therefore discussed to influence primary processes of the infection.

Appressoria formation during the pre-penetration process can be rated as a reaction to successful host recognition. Therefore a decrease in number of appressoria points to modified surface parameters that act as interactive mediators in this parasitic system.

In biotests different leaf surfaces from wild-type and various cuticular cer-mutants of barley are inoculated with fungal conidiospores. Distinct features such as chemistry, crystal structure and hydrophobicity of *H. vulgare* epi- and intracuticular leaf waxes are analysed and combined with germination rates of the conidiospores. Data are discussed in how far surface features of cuticular plant waxes are affecting the pre-penetration processes.

P1997. Supplement potassium to enhance foliar uptake of micronutrients

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Content of minerals in green leafy vegetables is essential for the nutritional and health status of the human body. To increase nutrients foliar application is an important and effective way. Burkhardt and Eichert 2001 showed that stomatal uptake of solutes increased with humidity, stomatal aperture and stomatal density. To determine the effect of potassium (2500g ha⁻¹) and two concentrations of B, Mn, Zn (M1: 150/250/210 and M2: 300/500/420g ha⁻¹) on the nutritional status of *Spinacia oleracea* the plants were sprayed 44 days after sowing in a field experiment with seven treatments and three replicates.

To study if a potassium application increases the stomatal width and consequently leads to an enhancement of mineral uptake stomatal conductance was measured.

The expected influence of potassium on stomatal conductance could not be proved.

The foliar application of M1 as well as the combined application of potassium and M1 did not affect the leaf concentration of micronutrients significantly whereas the doubled concentration, M2, increased the B content in leaves significantly and led to a slight rise of Mn, Zn content of spinach leaves was not affected.

P1998. The water permeability of cuticular membranes

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The water permeability of cuticular membranes (CM) isolated from the upper astomatous and lower stomatous surface of ivy (*Hedera helix*) leaves was measured by using a new method which allowed separation of total water flux through cuticle into its parts: the flux through pores and the flux through solid membrane. The principle of this method is based on fact that the diffusivity of water vapour in the gas phase can be manipulated by using gases with various molecular mass (e.g. helium, nitrogen, xenon) while diffusivity of water vapour in the solid phase is not affected. This approach allowed to calculate the flux of water across stomatal pores (stomatal transpiration) separately from the flux of water across the cuticle solid phase (cuticular transpiration) on stomatal CM. As we expected, water flux across the cuticle isolated from upper astomatous leaf surface was not affected by the gas composition. The water permeability of astomatous cuticles was about 7 times lower than water permeability of stomatous cuticles. Surprisingly about 80% of water flux was facilitated through solid (non-porous) phase of stomatous cuticle.

P1999. Leaf wax structures in selected Central European *Salix* species

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The morphology of the wax layer on the abaxial leaf surface was studied in 23 species of the genus *Salix*. A SEM analysis revealed that the lower part of the leaf blade can be covered by a nearly uniform wax film (*S. alpina*, *S. herbacea*, *S. pentandra*, *S. retusa* and *S. viminalis*) or specific wax structures (*S. acutifolia*, *S. alba*, *S. aurita*, *S. caprea*, *S. cinerea*, *S. daphnoides*, *S. elaeagnos*, *S. fragilis*, *S. helvetica*, *S. lapponum*, *S. myrtilloides*, *S. nigricans*, *S. purpurea*, *S. repens*, *S. reticulata*, *S. silesiaca*, *S. starkeana* and *S. triandra*). The above-mentioned structures (named here 'conicoids') are composed of apically convergent wax filaments. They did not appear on leaf margins and major veins. Size differences were found between some groups of species. Conicoids are formed by accumulation of new material at the base and simultaneous horizontal expansion due to leaf development. This causes the splitting of the originally continuous wax layer into patches. The observed differences between species can be used as additional features in classification of *Salix* species.

P2000. On the chemistry of *Selaginella*: Biflavonoids and unusual C₂₈-spirostene glycosides from Indian *Selaginella* species.

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Many aspects of the chemistry and the chemotaxonomy among the Lycopodiophyta (including Lycopodiales, Selaginellales and Isoetales) are still weakly elucidated. The lack of knowledge regarding secondary metabolites that could be used as markers in these groups makes a chemosystematic approach difficult.

During a survey of two Indian species, *Selaginella bryopteris* (L.) BAK. and *S. chrysocaulos* (HOOK. & GREV.) SPRING were investigated for their chemistry for the first time. We present herein data on biflavonoids of the amentoflavone- and hinokiflavone-type from these species, as well as the finding of steroids with C₂₁-carboxylation and unusual C₂₈-spirostene glycosides, which have not yet been described from any plant species. The classes of biflavonoids and steroids could, therefore, be useful chemotaxonomically among members of this taxonomic group. The chemotaxonomic relevance of the compounds within the Lycopodiophyta and related groups will be discussed.

P2001. Comparison of leaf flavonoids in *Crocus* species (Iridaceae) from Iran

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Crocus includes nine species in Iran. Intra- and inter-specific variation of population investigated among 38 populations. Flavonoids extracted from leaves, 2D-TLC and HPLC were used to separate those. Spots were observed using NP and four reagents. Data from HPLC scored by standardized peak area. Cluster analysis was carried out using squared Euclidean Distance and ward's methods. Grouping of the populations were interpreted comparing with geographical locality and altitudes. Some constituent were purified and identified at the level of classes of flavonoids by spectrophotometry and application of shift reagents. The main compounds were: flavon, flavonol, isoflavon and dihydroflavonols. Most of the flavonoids were flavon C-glycosides and some of flavonol O- glycosides. Luteolin, apigenin, methylated and hydroxylated of these observed repeatedly. Type of the flavonoids was similar at the level of intraspecies of *C. caspius* but their concentration was variable. Also, *C. cancellatus* populations were different with respect of type and concentration. Results show that *C. almehensis* and *C. caspius* were diverse from other by their flavonoid patterns.

P2002. Paralogy and homology in polyketide genes of lichen fungi

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Lichens are outstandingly rich in extralites of polyketide origin, both with regard to amounts produced and their structural diversity. While this has traditionally been used in the chemical classification of lichens, the analysis of natural thalli may still underestimate the chemical potential of the mycobionts. In axenic cultures, the fungi may produce a shifted spectrum of polyketides, and frequently also compounds that are unknown from the intact lichen. The goal of our investigation is to explore the genetic diversity of polyketide synthase genes in lichen mycobionts and to analyse these in a phylogenetic framework. The analysis of paralogous sequences will be used to assess which genes evolved prior or after the diversification of lichen lineages. The functional assignment of genes to particular compounds is nevertheless complicate, for this needs to consider incomplete sampling of paralogs and possible post-PKS modifications of compounds. The correlation of transcribed genes with produced compounds may result in a better estimate of gene functions. First results from these experiments are presented.

P2003. Characterization of different ecotypes of *Salvia officinalis* grown in Albania

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The study of reflectance signature of leaves, composition of essential oils and genetic polymorphism are applied to characterize some ecotypes of the species *Salvia officinalis* L. grown in Albania. Indicators like reflection values in some characteristic wavelengths (550 nm, 700 nm, 750 nm and 800 nm) and reflection ratios in these wavelengths have been determined via reflection spectra of leaves. The composition of the essential oil extracted from leaves by steam distillation, in two different vegetative phases, was determined via gas-chromatography and the identification of the peaks was based on their retention times. Genomic DNA isolated from young fresh leaves of individual plants were evaluated via RAPD analysis. The ecotypes collected in four different areas of Albania exhibited changes on the reflectance spectra and reflection ratios, differences on essential oil components especially in the quantity of thujons and camphor as well as showed a clear genetic variability. Based on the obtained results we conclude that these ecotypes can be grouped into clusters of similarity.

P2004. Flavonoids as characters in cladistic analysis of Lythraceae s.l.: use of biosynthetic pathway

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A coding method for using the steps of flavonoid biosynthesis as characters in cladistic analysis is presented. Eriodictyol intermediates the synthesis of flavones (luteolin) and flavonols (quercetin), while dihydrokaempferol intermediates only flavonol synthesis (kaempferol and quercetin). A cladistic analysis using all precursors as binary characters for flavonoid patterns observed in some genera of Lythraceae s.l. lead to the following relationship: (((((Heimia montana, Galpinia transvaalica) Punica granatum) Pleurophora anomala) Capuronia madagascariensis) ((Lythrum salicaria, Lagerstroemia tomentosa) Rotala ramosior) Woodfordia uniflora, Koehneria madagascariensis, Ginoria rohrii) G. nudiflora, G. glabra, G. americana, Adenaria floribunda). Species bearing quercetin derivatives, but no kaempferol, form a clade with luteolin bearing species. It suggests that those species produce quercetin via eriodictyol instead of via dihydrokaempferol. The results could suggest a new interpretation of flavonoid evolution. FAPESP, CAPES.

P2005. Two examples of correlation between alkaloid biosynthesis and phylogeny

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The biosynthetics involved in the production of plant metabolites are under evolutionary constraints. This means that they do not appear out of nowhere, and secondary metabolites are only retained if they give the organism that final edge of survival of the fittest. In systematics certain compounds has thus been used as taxonomic markers. Due to the hereditary nature of biosynthetic machinery a chemical can be regarded as a synapomorphy if it truly has arisen only once, thus defining a monophyletic group. The biosynthetic machinery necessary to produce colchicine and analogues appears to be a synapomorphy for the family Colchicaceae, eventhough the class of phenethylisoquinoline alkaloids are not. How unique a compound is may be due to only minor mutations in the machinery. Tracing the enzymatic paths might thus be necessary to ensure a synapomorphic status, as for the cyclopeptide alkaloids having different substructures in different systematical groups of eudicots.

P2006. Characterization of some *Thymus* taxa using molecular markers

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The similarity of *Thymus* species morphological and anatomical features poses a problem for adequate identification. The aim of our study is to be detect genetic diversity of some *Thymus* taxa,

based on their individual RAPD pattern. In the study RAPD markers have been used to provide more objective analysis of genotype and genetic relationships.

Materials and Methods: DNA from 8 Thyme taxa was isolated with Dneasy Plant Mini Kit (Quiagen) and analysed by randomly amplified polymorphic DNA (RAPD) with decamer primers (OPA, OPB, OPI, OPN, OPO, OPP, OPW) obtained from Operon Technologies (Alameda, CA.). Dendrogram was constructed based on the similarity matrix data by applying the unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis using the SYN-TAX 5.0 program package.

Results: among the examined 86 primers 54 showed polymorphic pattern. The most polymorphic pattern were shown by the following primers: OPA-3, OPI-7, OPI-12, OPN-(3-6), OPO-4, OPO-20, OPP-8. The number of bands obtained by primers varied from 11 (OPN-6) to 21 (OPN-4). On the basis of the observed bands it was possible to differentiate the taxa studied.

P2007. Comparative molecular and phytochemical investigation of Central European populations from *Leontodon autumnalis* (Lactuceae, Asteraceae)

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Previous analyses of *Leontodon autumnalis* L. revealed the existence of two chemotypes. In the current study, molecular and phytochemical methods were combined to investigate 24 Central European populations of *L. autumnalis*. DNA fingerprint profiles of 183 individuals were established by randomly amplified polymorphic DNA (RAPD) providing 77 molecular markers. Contents of phenolics and sesquiterpenoids of flowering heads and subaerial parts were determined by HPLC-DAD quantification. Evaluation of results by Principal Component Analysis (PCA) showed that geographic distribution of the two detected chemotypes partially overlapped. Phylogenetic groupings displayed in an unrooted Neighbour Joining (NJ) tree calculated from the RAPD data matrix correlated with the geographical origin of the plant material. However, genetic profiles were not consistent with the two chemotypes nor with the morphologically based subspecies of *L. autumnalis* recognized by some authors. In conclusion, morphotypes seem to be of multiple origin and chemical differences might be due to different ecological growing condition rather than genetically determined.

P2008. Chemodiversity of exudate flavonoids from the farina of *Dionysia* spp. (Primulaceae)

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Dionysia Fenzl comprises approx. 50 species, occurring mainly in mountain regions of Iran and neighbouring countries. Relationships to *Primula* subgen. *Sphondylia* are so close that *Dionysia* is likely to be incorporated in the genus *Primula* once the taxonomy is satisfactorily resolved. This relationship is also reflected by the production of farinose exudates in 9 studied *Dionysia* species. Their flavonoid composition corresponds largely to that known from farinose *Primula* species. The exudate is an almost pure mixture of several flavonoid aglycones. Chemodiversity features include a low degree of O-substitution and partly unusual substitution patterns. Unsubstituted flavone is an ubiquitous component of the flavonoid mixture, very often accompanied by 2'-hydroxy- and 2',5'-hydroxyflavones and their derivatives. Chalcones with corresponding substitution patterns are rather rare (*D. mira* only), whereas flavanones with different substitution patterns occur on most of the studied species. Chemosystematic aspects at the infrageneric and intrageneric level as well as ecological significance are briefly addressed.

P2009. Phytochemical studies of genus *Consolida* (DC.)S.F.Gray

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The Genus *Consolida* comprises 18 species in Iran. It has various from diterpens group. They divided in to two groups, C20-diterpen and C19 (Nourditepen). In this research we compare alkaloids in 3 species. Reproductive organs used for extraction. TLC on GF254 silicagel plates with solution system ethylacetate, Isopropanol,

Amonic 20% (45:35:15) used for alkaloid isolation. The spots observed under UV. In the TLC of flowers many similarity in *C. leptocarpa* spots and *C. leptocarpa* var. *khorsanica* were similar to *C. campocarpa* morphologically. This results proved by TLC investigation. This method also confirmed the differences between *C. rugulosa* and *C. leptocarpa*. The chromatogram of seed extracts of three species were studied. *C. leptocarpa*, *C. leptocarpavar* *khorsanic* and *C. rugulosa* showed similar pattern in TLC. They showed difference in flower alkaloids. TLC patterns in *C. persica* were completely different from other species. That shows the isolation of this species.

P2010. Comparison of essential oils and alkaloids in three species of *Annona* from Chiapas.

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The alkaloids and essential oils are a constant in the Annonaceae. However, few are the studies that integrate the chemical data with the plants physiology. In this research was compared the presence and distribution of alkaloids in *Annona lutescens*, *A. diversifolia* and *A. purpurea*, as well as the essential oils of their seeds. The plants were collected in the same locality of the state of Chiapas. The essential oils were analyzed by gas chromatography; the alkaloids were obtained by selective extractions of root, bark, leaves and seeds and the profiles was compared intra and inter specifically for TLC, the two major alkaloids were identified by spectroscopy as liriodenine and oxopurpureine. Were identified 22 similar essential oils in the endosperms of *A. diversifolia* and *A. purpurea* and 15 in *A. lutescens*. Were detected in *A. diversifolia* and *A. purpurea* 23 alkaloids and 11 in *A. lutescens*. For the presence of essential oils and alkaloids *A. diversifolia* and *A. purpurea*, had a chemical relationship, in agree with the physiologic likeness exists when fructification time and to present seeds with latency.

P2011. Allelopathic effects of *Artemisia* on seed germination and growth of *Hordeum vulgare* L. and *Agropyron*.

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The allelopathic potential of *Artemisia herba-alba* on germination and growth of *Hordeum vulgare* L. and *Agropyron tauri* was investigated. Barly as a crop plant and *Agropyron* as a rangeland plant were selected.

Effect of root, stem, leaf and flower of *Artemisia* was investigated separately.

These experiments were carried out using completely randomise block design. From the first aqueous extract (10% W/V) solution with 5, 10, 20, and 40 percent were obtained. Then the effects of these solution on germination and growth of barly and *agropyron* were studied. Also the effects synthesized santonin with concentration of 20, 40, 80 and 160 ppm and *Artemisia* flower powder with amounts of 0.2, 0.4 and 0.8 g were investigated.

The observation showed that extracts of leaf and flower statistically reduced the seed germination and growth of barly and *Agropyron*. However the extracts of root and stem did not showed significant reduction. Also the santonin with concentration of 80 and 160 ppm and flower powder reduced seed germination and growth of barly and *Agropyron*.

P2012. Dynamics of peroxidase complex activity in pathogen system *Claviceps purpurea* - *Secale cereale*

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The strains of fungus have different biosynthetic alkaloid capabilities and predominantly produce a certain peptide alkaloid. In submerged cultures enzyme activity is lower at ergocristine strains and higher at cultures obtained from ergotamine and ergocryptine sclerotia. Peroxidase activity of sclerotia is strong at beginning of ontogenetic development, and decrease to the end of sclerotia development. The parasite influences peroxidase activity in host plants. The alkaloid accumulation in sclerotia and intensity of peroxidase activity there are in relation of inverse proportionality. The alkaloid complex influences the peroxidase activity in plant. Comparatively with control, enzyme activity level is

lower in parasitized plants by ergotamine sclerotia and higher in parasitized plants by ergotamine sclerotia. Peroxidase isoforms number in host plants varies depending on age and predominant alkaloid in sclerotia which growth on *Secale cereale*.

P2013. Use of repellent plants in malaria vector control

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A review of literature indicated that 32 plants which belonged to 13 genera in 20 families have been investigated for repellent properties against the malaria vector mosquitoes. Owing to the prevalence of malaria in tropical Africa and other tropical regions of the world, repellent plants have found extensive usage in various forms for mosquito control, and thereby complement other methods of managing the malaria burden. Direct burning, placing whole potted plants in houses and local application of plant juices on the skin are common traditional methods employed especially by the rural dwellers in exploiting the repellent properties of promising plants. Protection periods of repellent products are usually short, but methods for improvement have been developed. Base properties of formulated essential oil repellents have been modified to improve efficacy and reduce side effects on human subjects. Bioactive repellent compounds have been isolated and notable ones identified.

P2014. Comparative research involving macro- and microelements in pathogen system *Claviceps purpurea* - *Secale cereale*

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In the pathogen system *Claviceps purpurea* - rye, the parasite produces profound modifications. In order to study the alkaloids' influence upon the mineral metabolism we have investigated the some macro- and microelements in whole rye plants parasitized by *Claviceps purpurea*, as well as from sclerotia formed on these plants. These elements are found in different amounts, depending on the alkaloids which act upon the plant. There is a convergence of the manifestations for the calcium ions, depending on the predominant alkaloid which acts upon the plants. In this pathogen system, mineral metabolism is strong influenced by bioproductive properties of different alkaloid strains of parasite both in the host plants and in ergotamine, ergocristine or ergocryptine sclerotia. The ergot alkaloids influence rye plants' metabolism, by their concentration and alkaloid type produce modifications of some ions concentration.

P2015. Specific impact of changed microgravitation upon pigment composition reaction against viral infection.

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Comparison of two abiotic factors - viral infection (virus of wheat strip mosaic) and changed microgravitation (by clinostat rotation) showed their antagonistic impact upon pigment composition of Apogej wheat variety (created for the space flight specially). Virus of wheat strip mosaic in conditions close to natural (unmoving plants without containers) caused total chlorophyll and carotenoid content in Apogej wheat variety decrease approximately by 20-30 %, chlorophyll/carotenoid ratio decreased by 10 %. In microgravitation conditions infected plants showed opposite to control pigment complex specific reaction. Main pigment content increase in viral infected plants compared with those treated by microgravitation only was observed. Pigment content in wheat leaves correlate with crop productivity of microgravity variants. Mechanism of microgravity adaptive reaction with/without virus infection discussed.

P2016. The Essential Oil Composition of Some *Salvia* Species from Bosnia

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The genus *Salvia* L. (Lamiaceae) comprises about 900 herbs and shrubs widespread throughout the world, and is represented by 14 species in Bosnia and Herzegovina, some of them being endemic. Air-dried plant material of *S. officinalis*, *S. glutinosa*, *S. verticillata* and *S. pratensis* was subjected to different methods of extraction. The essential oils were analysed by GC-MS.

alpha-Thujone and beta-thujone constituted up to 65.04% and 7.65%, respectively, of total headspace extractives of *S. officinalis* essential oil, while in steam distilled oil dominant constituents were manool (48.10%) and viridiflorol (31.14%).

Ethylhexadecanoate (10.8 %) and tricosane (10.7%) were main components of *S. glutinosa* oil.

Content of germacrene D (4.1 - 20.3%), beta-caryophyllene (9.4 - 20.0%) and spathulenol (7.0 - 20.0 %) varied for different plant parts of *S. verticillata*.

S. pratensis essential oil was characterised by spathulenol (32.9 %) and caryophyllene oxide (12.5%), while its endemic variety *S. varbossania* K. Malý contained caryophyllene oxide (19.4 %) and beta-caryophyllene (9.6 %) as the major components that permit the differentiation between these species.

P2017. Early Flowering Response of *Dendrobium Sonia* 'Eiskul' and Expression of Some cDNA Induced by Chitosan

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Six types of chitosan molecules, polymer form with 70% degree of deacetylation (DD)-P70, 80% DD-P80, and 90% DD-P90, together with oligomer form with the similar %DD-O70, O80 and O90, were tested for the effects on growth of *Dendrobium Sonia* 'Eiskul'. The various concentrations of chitosan at 0, 1, 10, 50 and 100 ppm were added to the fertilizer solution and applied once a week during the experiment. No significant effects of chitosan on vegetative growth of 1 year-old after exflasking orchid plants were detected during three months of application. However, early flowering response was found best induced by O80. The differential display of gene expression of O80 chitosan-treated orchid shoot and non-treated control showed the total of 145 different cDNA bands. Some of the cDNAs were cloned. Sequence analysis of these cDNA clones indicated their possible roles in calcium signaling and floral development. The data supported the high potential use of chitosan as plant growth stimulator.

P2018. Oil and fatty acid composition of species with potential use pharmacology of semi-arid northeastern

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Oil obtained from seeds at seven species with potential use pharmacology, for will be tested in the etiological agents of several endemic disease were analyzed for total lipids as well as fatty acids. The oil was extracted by *n*-hexane, saponified with methanolic NaOH and methylated with methanolic Boro trifluoride. The fatty acids were analysed in a Shimadzu 5017-A gas chromatography (GC) and MS-Mass Spectrometry. Total oil content ranged from 8,0% in *Ipomoea incarnata* Meisner in Martius to 21,0% in *Leonotis nepetaefolia* (L) R. Br. Palmitic was the main acid in *Sapim glandulatum* Pax (42,5%) and *Leonotis nepetaefolia* (47,9%), while oleic acid was found in high quantity in *Mansoa hirsuta* DC. (62,4%). *Myracrodruon urundeuva* Fr. All..showed highest amount (67,2%) of fatty acids not identified, followed of the *L. nepetaefolia* (10,4%), *S. glandulatum* (8,35%) and *M. hirsuta* (3,3%), probably a unusual fatty acids. The occurrence of the unusual fatty acids to wear a expectation to find bioactives.

P2019. A new sesquiterpene from *Centaurea cadmea* Boiss.

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The large genus *Centaurea* L. (Asteraceae) comprises about five hundred species which are predominantly distributed around the Mediterranean area and West Asia. *Centaurea* species are used for many purposes such as stimulant, tonic, anti-diabetic, diuretic, cholagog and anti-inflammatory agent in traditional medicine. The phytochemical studies on this genus deal mainly with sesquiterpene lactones. *Centaurea cadmea* Boiss. is an endemic species for Western Anatolia. The plant is not used in Turkish folk medicine; however, other species of the same genus (*C. pulchella* C. *drabifolia* and *C. solstitialis* subsp. *solstitialis*) are used for treatment of abscess, in hemorrhoid and for colds. Our ongoing study resulted in the isolation of a new sesquiterpene (Eudesma-4(14), 11(13)-dien-12-oic acid, 2, 6 -dihydroxy-, -lactone) from the chloroform extract of aerial parts of *C. cadmea*. The structure elucidation of the new compound was achieved by a combination of one- (¹H, and ¹³C) and two dimensional (G-HMQC, G-HMBC, COSY and NOESY) NMR techniques.

P2020. The dynamics of some biochemical and physiological parameters at some *Pelargonium* species producing of essential oils with microbiological effects

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We have been studied three *Pelargonium* species (*P. zonale*, *P. radens* and *P. odoratissimum*) and have investigated assimilating pigments, fat, respiration, sugars from leaves and essential oils content from herba. The fat content is in inverse ratio to the essential oils extraction efficiency and to the number of components, for all species. At *P. zonale* there is a very big difference between the number of components before the flowering period (49) and during the flowering period (161). The physiological and biochemical parameters investigated have been found to have various values and there is a correlation between physiological parameters and essential oil content; the dynamics of these parameters was found to be various from one period to another, as well as from one species to another. Microbiological tests for essential oils produced by *P. radens* evidence a higher inhibition effect upon *Staphylococcus aureus* then *Escherichia coli*.

P2021. Phytochemical investigation of *Rotala ramosior* (L.) Koehne and *Ammannia coccinea* Rottb. (Lythraceae) from the Southeastern United States

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The landmark work by Robert Hegnauer, *Chemotaxonomie der Pflanzen*, promoted interest in the fields of phytochemistry and chemosystematics. The family of Lythraceae has been widely neglected in phytochemical research, partially because only few species of Lythraceae have records of ethnomedicinal use.

As part of our research on the phytochemistry of plants of the Southeastern United States, we examined the chemistry of two herbaceous species of Lythraceae. These species, native to large parts of North America, *Rotala ramosior* (L.) KOEHNE and *Ammannia coccinea* ROTTB., are commonly found in ditches and moist agricultural land, where they have a potential to be weedy as with *Rotala indica* KOEHNE, which is a noxious weed in rice fields in Southeastern Asia.

Results from the examination of the non-polar extracts of aerial flowering plant material are presented and implications in relation to known chemistry of the family are discussed.

P2022. Effects of flavonoid-rich methanolic extract of *Cnestis ferruginea* DC. (Connaraceae) on permeability transition of isolated rat liver mitochondria

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Changes in mitochondria matrix volume resulting from opening of the mitochondria transition pore triggered by 300nM Ca²⁺mg⁻¹ protein was quantified by decrease in absorbance at 540nm.

There was little or no change in matrix volume of mitochondria (0.000 - 0.002) in the absence of a triggering agent. However, triggering agent (Ca²⁺) opened the mitochondrial permeability transition pore making the inner membrane non-selectively

permeable to small solutes resulting in a profound increase in matrix volume (0.000 - 0.400). 0.1mM spermine caused inhibition of permeability transition (0.000 - 0.050). 10µg, 20µg and 30µg of methanolic extract of *Cnestis ferruginea* inhibited the permeability transition to a larger extent, 0.013 (1min), 0.035 (12min), 0.008 (11min) respectively. 20µg was anomalous in its effect, as it did not generate the maximum likelihood inhibition compared to 10µg and 30µg. *Cnestis ferruginea* may delay apoptotic cell death.

P2023. Bioprospection of Brazilian semi-arid land flora

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The semi-arid region of NE Brazil, covering 975,000 km², supports a population of c. two million inhabitants. The region is characterized by its low socio-economic index, high level of environmental degradation and low level of knowledge of its biodiversity. The Project on the Semi-arid, of the Instituto de Milênio, aims to trace the chemical profile of species with biologically active compounds, which occur in the semi-arid and with this in view, collections of over 2000 species of native plant were assembled. 90 of these, principally species of Leguminosae, Solanaceae, Euphorbiaceae and Labiatae were pharmacologically evaluated in terms of nitric oxid production inhibitory activity, linfoproliferation inhibition, and imunomodulation. From a total of 536 extracts, 103 pure compounds were isolated, of which 14 were previously unknown, and 20 semi-synthetic derivatives were obtained and pharmacologically evaluated. These compounds are new potential medicines for diseases typical of the semi-arid region. The species are also been studied with a view to their propagation, molecular and genetic characterization and conservation.

P2024. Essential oil from the flowers of *Edgeworthia chrysantha* Lindl

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Edgeworthia chrysantha (family Thymelaeaceae), native to China, is a deciduous small shrub. It has strongly aromatic flowers. It is used as folk medicine to cure eye disease in China. The flowers are reputed to cure wet dream and abnormality of leucorrhea. Ethanolic and anhydrous extracts of *E. chrysantha* also showed antibacterial and antifungal activities. Some chemical components such as coumarin and glucoside have been studied.

The flowers of *E. chrysantha* afforded 0.13% (of fresh wt.) essential oil, characterized by a special odour. A total of 45 compounds were identified by GC-MS, about 75.75% of the composition of the oils. Chemical structures of compounds are given by NIST(2004) database. The oil is rich in ester (41.4%) and ketones (16.57%). The remaining portions of the oils consist of long chain alkane (6.07%), alkyl aromatics (4.76%) and other trace amounts components.

P2025. The effects of hydro alcoholic extract of soya in the histological structure and the level of estradiol and testosterone in male sorie

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Soja is a rich source of phytoestrogen. soja extract can change the structure of male reproductive tissue and it can influence on the level of sex hormon. For this purpose, 50male sories were selected and divided into 4 groups. 3experimental groups were fed with 150,100 and 75 mg/kg of hydro alcoholic extraction of soja and control group were fed with the solvent (water) for 50 days. Blood samples were taken in zero and fiftieth day of experment and the level of sex hormon were measured. Histological of servtions were revealed some changein testis. In the testis many spermatocytes at the metaphase stage in 150 and 100 mg/kg treated groups and some of these mitotic figures, most of the spermatocytes is denaturing of DNA strands. In conclusion,

it seems that soja extract can change the spermatocyte cyto architecture, in low concentration, it may induce proliferation and high concentration, it may lead to cell death and nucleus fragmentation. The result indicated that level of testosterone and estradiol was also decreased significantly in the groups, which treated with 150mg/kg and 100mg/kg.

P2026. Hepato Protective and Antioxidant Role of *Berberis Tinctoria* Lesch on Paracetamol Induced Hepatic Damage in Rats

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The scientific evaluation of medicinal plants used in the preparation of folk remedies has provided modern medicine with effective pharmaceuticals for the treatment of diseases. This prompted us to the investigation of Hepatoprotective activity of *Berberis tinctoria* Lesch leaves. The methanol extract of the leaves of *Berberis Tinctoria* Lesch (Berberidaceae) was evaluated for Hepatoprotective activity in rats by including acute damage by paracetamol (750mg/kg). Hepatoprotective activity was measured by using biochemical parameters such as serum transaminase (SGOT and SGPT), alkaline phosphatase (ALP), bilirubin and total protein. The methanol extract of *Berberis Tinctoria* (MEBT) (150mg/kg and 300mg/kg) produced significant hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin and lipid peroxidation while it significantly increased the levels of GSH, CAT and Protein in a dose dependent manner. The effects of MEBT were comparable to that of standard drug Silymarin. These results suggest that *Berberis Tinctoria* may be potential therapeutic value in some liver disorders.

P2027. Fatty acid composition from cypselas of some *Centaurea* species (subgenus *Jacea*)

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In this work fatty acid composition from mature cypselas lipids of *Centaurea jacea* L., *C. phrygia* L., *C. nervosa* Willd., *C. triumfetta* All., *C. cyanus* L. and *C. montana* L., belonging to subgenus *Jacea*, were investigated. The specimens were collected at natural habitats in Serbia and Montenegro. The fatty acids were extracted and prepared by standard procedure. They are analyzed by means of GC and identified comparing to authentic standards. Four different fatty acids were determined: palmitic, stearic, linoleic and linolenic. All of investigated species were characterized by presence of all identified acids. Results showed that the linolenic was predominant acid in all investigated species. The obtained amounts of linolenic fatty acid were following: 66.9 % *C. jacea* L., 73.1 % *C. phrygia* L., 35.8 % *C. nervosa* Willd., 60.6 % *C. triumfetta* All., 70.7 % *C. cyanus* L. and 59.2 % *C. montana* L. Stearic acid was present in traces in *C. jacea* and *C. nervosa* seeds. Given results revealed some differences in fatty acid composition of the analyzed species. Taxonomic significance of fatty acid composition of this group and other *Centaurea* spp. from previous works is discussed.

P2028. Pentacyclic triterpenoid from the leaves of *Ficus racemosa* and its anti-inflammatory activity

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Traditional medicines, with a very long history and remarkable features, are very popular in Asian countries. Phytochemicals in leaves, fruits, vegetables and traditional herbal medicinal plants have been found to play protective roles against many human chronic diseases including cancer, cardiovascular and inflammatory disorders. Among the thousands of phytochemicals found in the traditional medicines, steroids and triterpenoids stand out as two most important compounds. A single plant could contain highly complex profiles of these compounds. This makes the separation and detection of these phytochemicals a challenging task. The present review focuses on the detailed study of isolation and characterization of lupeol acetate, a pentacyclic triterpenoid

from the petroleum ether extract of the leaves of *Ficus racemosa* L. Syn. *Ficus glomerata* Roxb. (Moraceae). The anti-inflammatory activity of this compound has been evaluated and found to be effective in both acute and the chronic models in rats.

P2029. Fruit maturation effect on hesperidin content in some local *Citrus* varieties in north of Iran

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Hesperidin is a flavonoid compound that found in *Citrus* fruit peel. In view of the fact that, hesperidin content depends on fruit growth and development process, it is necessary to determine the best time of fruit harvesting for optimal hesperidin content. In order to determine the best time of fruit harvest from hesperidin content point of view in four varieties of *Citrus* including, local sweet orange, bloody orange, sour orange and local tangerine this study was carried in north of Iran. Harvest times of fruit were consist of 45, 50, 55 and 60 days after full bloom and a comparison also was performed between fruits that were fallen below trees and those on trees. Results showed that hesperidin content was increased in the varieties with progress of growth and development process of fruit. Best profitable time of harvest was 55 to 60 days after full bloom. There wasn't significant difference between fallen fruits and those on trees from hesperidin content point of view. *Citrus* varieties had different hesperidin content.

P2030. Some Lichens of Ethnic Value from Darjeeling Himalayas of India as Antimicrobial Agents.

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Darjeeling hills are located on the Singalila range of Eastern Himalayas bordering Nepal and Sikkim. The tribes here traditionally use extracts of various lichens for curing several ailments ranging from cuts and burns to gastro-enteric disorders. For this study, ethanol extracts of three species of lichens namely *Usnea* sp., *Ramalina* sp. and *Parmelia* sp. were fractionated with water and methanolic ethyl acetate respectively, for obtaining non-polar and polar compounds like glycosides and flavonoids. The methanol fraction of *Usnea* sp. revealed three bands, of which the major band showed significant presence of reducing sugar. When tested for antimicrobial activity against a range of pathogenic organisms, this fraction appeared to be highly potent. Effects on population growth as well as inhibition of protein synthesis and growth were studied. The gram-positive *Staphylococcus aureus* proved to be most sensitive. Comparable results were observed when the cells were treated with the broad-spectrum antibiotic ampicillin. Gram negatives like *E. coli* were more resistant to fractions of *Ramalina* sp. and *Parmelia* sp. had no significant effect.

P2031. Terpenoids from Juniperus plants

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In a survey of chemical components from useful plants grown in western North America, we have identified a number of various types of phenolic compounds (phenylpropanoids, neolignans, and flavonoids) in their glycosides from *Juniperus communis* L. var. *depressa* Pursh and *J. occidentalis* Hook. (Cupressaceae) collected in Oregon, USA and these chemical results have been reported already. Upon continued chemical investigation of these *Juniperus* plants, two new monoterpenic glycosides and three new natural megastigmane glycosides were isolated along with a known megastigmane glycoside, (6S, 9S)-roseoside A. Their whole structures were determined on the basis of spectral (MS, ¹H- and ¹³C-NMR) by using 2D-techniques, and CD) and chemical evidence. Essential oil compositions of both *Juniperus* plants were also examined. In addition, antibacterial activities of the isolated components against *Helicobacter pylori* were also investigated. As a result, one of the components showed a potent inhibition comparable to that of a positive control.

P2032. Preliminary screening of *Leucosidea sericea* (Rosaceae) for biological activity and the isolation of anthelmintic and antiplasmodial compounds.

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Leaves of *L. sericea*, an aggressive invader of disturbed areas, are used medicinally by indigenous South African tribes as a vermifuge and astringent. Extracts of the dried leaves, prepared using solvents of different polarities, were screened for bioactivity. Antibacterial activity was investigated by serial dilution to determine the Minimum Inhibitory Concentration (MIC). Ethyl acetate and methanol extracts exhibited the highest inhibitory effect against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* with MIC values between 0.31 - 0.63 mg/ml and 0.078 - 0.31 mg/ml respectively. Bioautography of the more non-polar extracts indicated zones with antibacterial activity. Bioassay guided fractionation of these extracts and subsequent chromatographic purification procedures yielded pure compounds, identified as phloroglucinol derivatives using GC-MS and NMR techniques. According to literature these compounds have anthelmintic and anti-plasmodial properties.

P2033. Antifungal activities from tropical sawdust hardwoods

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Tropical sawdust hardwoods of *Hopea odorata* Roxb., *Shorea albida*, *S. obtusa* Wall ex Blume, *Tectona grandis* L.f. and *Xylia xylocarpa* Roxb. were extracted by CHCl₃-MeOH. Thirteen strains of wood rot fungi were tested with these extracts. We found that *T. grandis* inhibit growth of brown rot fungi, *Gloeophyllum trabeum* and *Gloeophyllum sepiarium*, as well as white rot fungi, *Phlebia brevispora* and *Merulius tremellosus*. In order to learn more about the possible mode of action, the effect of *T. grandis* extract on an *Aspergillus niger* was studied. This particular cell wall damage model will show induction of 1,3 -D glucan synthase by coupling a green fluorescent protein (GFP) marker encoding sequence to the glucan synthase gene. Induction will be detected as fluorescence in the fungal cells. The results showed that *T. grandis* extract induced fluorescence in the transgenic *A. niger* cells which indicated that the plant extract contains compounds that damage the fungal cell wall.

P2035. Trichome morphology and chemical aspects of the essential oil of *Lippia scaberrima* Sond. (Verbenaceae)

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Lippia (Verbenaceae) comprises about 200 species occurring mainly in tropical and temperate regions of America and Africa. Six species are found in savanna and grassland areas of southern Africa. *L. scaberrima* is a medicinal, aromatic shrub, invasive in overgrazed land.

Trichome morphology, distribution and density, and oil localization was investigated using light microscopy and SEM. Essential oil, extracted from a bulk sample by steam distillation, was characterised by GC-FID and GC-MS. The effect of post-harvest drying on trichome structure, as well as on essential oil yield and composition was determined. In a consecutive study, the chemical profiles of individual specimens, harvested within a single and different localities, were compared.

The major oil components were identified and include limonene and R-carvone. Post-harvest drying was found to increase the quantities of extractable oils. Combined with SEM results, it was proved that glandular trichomes are not as susceptible to oil loss through desiccation, as previously suggested.

P2036. Essential oil composition from the different parts of *Eryngium glaciale* Boiss. from Spain

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The *Eryngium* L. genus belongs to the Apiaceae family and, with about 250 species, has a cosmopolitan distribution. In the Iberian Peninsula grow 14 of the 26 species described in Flora Europaea. The essential oil from the different parts (stems + leaves, inflorescences and roots) of *E. glaciale* Boiss. gathered in Sierra Nevada (Spain) have been extracted by steam distillation and analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). Quantitative but not qualitative differences have been found between the analysed parts. The principal compounds from the inflorescences oil were found to be phyllocladene isomer (43.5%), (E)-Caryophyllene (15.2%) and Valencene (11.5%), while the oil from stems and leaves only showed phyllocladene isomer (41.3%) as main one. The oil from the roots presented phyllocladene isomer (49.4%) and linalool (19.1%) as major constituents. As far as we know this is the first report about the essential oil composition of *E. glaciale*. It is worth mentioning the presence of a diterpenes as main compounds of the essential oil.

P2037. Antifungal activity of *Achillea millefolium* L. essential oil

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The essential oil of *Achillea millefolium* L. as potential antifungal agent was analyzed. The main compounds in the oil were 1.8-cineole (28.08%), camphor (10.57%), p-cymene (8.29%), -terpinene (6.60%) and -thujone (5.73%). Following micromycetes as test organisms were used: *Alternaria alternata*, *Aspergillus* spp., *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *C. fulvium*, *Fusarium tricinctum*, *F. sporotrichioides*, *Mucor mucedo*, *Penicillium funiculosum*, *P. ochrochloron*, *Phomopsis helianthi*, *Phoma macdonaldii*, *Trichoderma viride*, *Trichophyton menthagrophytes* and yeast *Candida albicans*. Using the microdilution method, minimum inhibition concentrations (MIC) and minimum fungicidal concentrations (MFC) were recorded. Commercial antimicrobial bifonazol was used as a control. The concentrations of 15 µl/ml of essential oil showed fungicidal activity against *Aspergillus*, *Penicillium* and *T. viride*. For *Mucor mucedo* MFC was 10 µl/ml, and for *A. pullulans* 5 µl/ml. Fungistatic and fungicidal activity of the oil for the other tested micromycetes were 1-2.5 µl/ml.

P2038. One new triterpenoid saponin from *Meyna laxiflora*, a less known medicinal plant species used for antifertility

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Meyna laxiflora Robyns (family Rubiaceae) is a small tree and found wild in North East India. Seeds of *M. laxiflora* are reported to induce termination of pregnancy and in some cases permanent sterility in women. Fruit pulp is also used as contraceptive. Leaves are considered to be good for diphtheria and used as antidandruff agent. In continuation of our search for plant based antifertility agents we have carried out a preliminary screening of various extracts of seed, fruit pulp and leaf of *M. laxiflora*. The alcoholic extract of the defatted seeds responded to the characteristic colour test for triterpenoid saponin. As potential antifertility agents from plants include triterpenoid saponin, it has prompted us to isolate and characterize the triterpenoid saponin of this plant. The crude saponin was precipitated from the concentrated ethanolic extract using a standardized procedure. Column chromatographic separation of this mixture yielded 3,19 -dihydroxy-urs-12-en-24,28-dioic acid-28 -D-glucopyranoside (I) along with other compound which are still under investigation. Saponin (I) has been characterized by both chemical and spectroscopic methods.

P2039. Alkaloids in calyx and corolla of *Erythrina coralloides* A.DC. and *Erythrina americana* Miller (Fabaceae).

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In Mexico the flowers of *Erythrina* species are used to cook, but their use is limited because the toxicity. The objective was to determine the alkaloid content in calyx and petals in *E. coralloides* and *E. americana* using liquid chromatography coupled with mass

spectrometry (LC/MS). Crude alkaloid fractions were prepared. Identification of the alkaloidal extracts was done by LC/MS. Was observed differences between calyx and corolla in *E. americana*. In the calyx -erythroidine, -erythroidine, 8-oxo-erythroidine, erythrinine, erythraline and crystamidine was observed, whereas in corolla -erythroidine, -erythroidine, 8-oxo-erythroidine, erythrinine and erysodine. In *E. coralloides* calyx was found the same alkaloids than *E. americana* calyx and also erysodine and 8-oxo-erythraline. In corolla the same pattern than in the calyx was observed but less 8-oxo-erythroidine. The method of LC/MS allowed the detection of alkaloidal structures in calyx and corolla of *E. americana* and *E. coralloides* that had not been identified in previous studies.

P2040. Antimicrobial evaluation of extracts of *Tillandsia imperialis* (Bromeliaceae).

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Tillandsia imperialis is used in Mexican folk medicine mixed with other plants and animals for respiratory ailments. Six crude extracts: hexane (H), Methylene Dichloride (MD) and methanol (M) from leaves and inflorescence were evaluated for antimicrobial activity against bacteria and one yeast. The minimal inhibitory concentration (MIC) was determined for each extract using a two-fold dilution assay.

Five extracts exhibited antimicrobial activity against at least one of the microorganisms tested at concentrations of 8 mg/ml or below. Three extracts inhibited *Staphylococcus aureus*: the H extract from the leaves (MIC 4mg/ml); the (H) and (MD) extracts from inflorescence (MIC 0.5mg/ml). *Streptococcus faecalis* was inhibited by (H) inflorescence extract (MIC 8mg/ml). *Klebsiella pneumoniae* and *Salmonella typhi* were inhibited by (M) extracts from leaves and inflorescence (MIC 8mg/ml). Neither one of the extracts showed antimicrobial activity against *Candida albicans* and *Escherichia coli*.

This indicates the existence of antimicrobial compounds in the extracts and show a correlation between the traditional uses of this plant and the experimental data.

P2041. Alkaloid composition of *Erythrina americana* Miller and *Erythrina coralloides* A.DC seeds

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In Mexico an important number of *Erythrina* species is found. Many of them are used in agro forestry systems. As typical legumes improve the soil and provide animal fodder, medicines and wood products. These attributes are diminished by the toxicity of the plants because they accumulate alkaloids particularly in the seeds. This work is an investigation of *E. americana* and *E. coralloides* seeds about their alkaloid composition using liquid chromatography-mass spectrometry as analytical tool. The crude methanolic extracts of both species showed some differences related with the alkaloid profile. In the seeds of *E. americana* some dienoic alkaloids such as erythraline, erythrinine, crystamidine and 8-Oxo-erythraline and the lactonic alkaloids -erythroidine and -erythroidine dominate. In contrast, the alkaloid content of *E. coralloides* is observed to be mainly erythrinine, crystamidine and 8-Oxo-erythraline in lesser amount. According to these data erythrinine was found regularly in the seeds of *E. coralloides* whereas in *E. americana* it was scarce. However, the presence of the lactonic alkaloids were dominant in *E. americana* and scarce in *E. coralloides*.

P2042. Phytochemistry and biological activity of root and leaf extracts of *Aspidosperma polyneuron* from Londrina, Brazil

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The genus *Aspidosperma* (Apocynaceae) has been well studied but there has been little investigation on the roots of *A. polyneuron*. This paper shows this species as a pharmacological potential using both roots and leaves extractives, which were subjected to bioassay with *Artemia salina*, using a modified Meyer method. The LD₅₀ were 69 and 36 ppm for the roots and leaves, respectively

(extracts were considered active at LD₅₀ <1000 ppm). The extracts were also tested against eight yeast and fungal species by the diffusion test, and did not inhibit their growth. The compounds were identified by chromatographic and NMR, GC-MS, IV techniques. From the roots were isolated: aspidospermine, lupeol, aliphatic alcohols and hydrocarbons of long chains, β-sitosterol, stigmasterol, and one quebrachamine derivative, whose structure is being investigated. From the leaf extracts were isolated: lupeol, ursolic acid, oleic acid, linoleic acid, carboxylic esters of long chains and a cyclitol. Some of these compounds quoted above have been described to possess pharmacological properties in laboratory animals, such as antiinflammatory, antiplasmodial, and others.

P2043. Evaluation of essential oil composition of *Pterospartum tridentatum* grown in Portugal

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Pterospartum tridentatum L. Willk. [= *Chamaespartium tridentatum* (L.) P. Gibbs.; *Genistella tridentata* (L.) Samp.] is an European endemic Fabaceae (= Leguminosae) species. This small shrub, up to 100cm, is very common in the mountains of the north-centre of Portugal showing yellow flowers, alternate branches and coriaceous winged stems. The flowers are traditionally harvested in spring and either used as condiment or in traditional medicine. The infusion of the dried flowering tops is used as an excellent bechic and emollient.

The essential oils, isolated by hydrodistillation and distillation-extraction, from the aerial parts of different populations from *P. tridentatum* collected during the flowering and vegetative phases, were analysed by GC and GM-MS.

All the *P. tridentatum* populations studied afforded a yellowish oil in a yield of <0.05% (v/w).

cis-Theaspirane, trans-theaspirane and octen-3-ol were, in variable amounts, the dominant components of the oils. Theaspiranes are common flavouring agents responsible for the tea, herbal, green and slightly spicy odour of the plant.

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P2044. Novel ribosome inactivating proteins (RIPs) from Himalayan *Viscum album* (L.)- potential biomolecules for bioprospecting

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Ribosome inactivating proteins (RIPs) of *Viscum album* constitute the active principle of traditional tumor therapy in Europe. Himalayan *V. album* populations showed high morphological diversity. To assess the prospecting potential of this wild resource, 4 novel isoforms of its RIP (HmRIP) were purified and characterized. HmRIP was stable to a broad range of temperature (0-65 °C) and pH (3-9). Unlike all other type II RIPs, HmRIP1, 2 and 4 showed unique sugar affinity for L-rhamnose, meso-inositol and L-arabinose besides the galactose. Amino acid sequence of HmRIP determined by cDNA cloning showed deletions at one of the two sugar-binding sites. Crystal structure of HmRIP-sugar complex determined by X-ray crystallography revealed a significantly different conformation of the 2 sugar-binding pocket. Due to deletion of two critical residues forming wall of the pocket, the site has become much broadened. Therefore the sugar binds in a significantly different manner. The recognition of the sugar chains present on the cell surface is the first step in biological activity therefore HmRIP with distinct sugar specificity has pharmacological significance.

P2045. Phytochemistry of *Riccia nigerica* jones

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The Phytochemical composition of *Riccia nigerica* Jones have been studied. Fresh plants of *Riccia nigerica* were extracted using acetone. Fractions were obtained through column chromatography. The absorbance spectra of the five fractions of the extracts showed a fluctuation from 0 to 1.18 at 400 to 950 wavelength. The anti-microbial activities of the crude extracts were tested against some selected species of bacteria and fungi, thus the test extract was found to have significant inhibitory effect (>17mm) as indicated by zones of inhibition on *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* sp. The results of the phytochemical study outlined in this work has shown that the extract of *Riccia nigerica* exhibited a remarkable inhibitory effect on test organisms both bacteria and fungi.

P2046. The Needle Volatile Composition of Trispecies Hybrids (*Pinus nigra* J. F. Arnold x *P. sylvestris* L.) x *P. densiflora* Siebold et Zucc. and (*P. nigra* J. F. Arnold x *P. sylvestris* L.) x *P. thunbergiana* FrancoM. Idzajt¹, H. W. Pfeiffer², M. Zebec¹;¹University of Zagreb, Faculty of Forestry, Zagreb, Croatia, ²University of Graz, Institute of Plant Physiology, Graz, Austria.

The needle volatile composition of two trispecies hard pine hybrids produced by the controlled hybridization and their parents was researched with gas chromatography and gas chromatography/mass spectrometry in order to explore the utility of terpenes in hybrid identification as well as confirmation of hybridity. The analysed trispecies hybrids were: (*Pinus nigra* J. F. Arnold x *P. sylvestris* L.) x *P. densiflora* Siebold et Zucc. (= *nisyde*) and (*P. nigra* x *P. sylvestris*) x *P. thunbergiana* Franco (= *nisyth*). The female parent was F_1 hybrid *P. nigra* x *P. sylvestris* (= *nisy*). The male parents were *P. densiflora* (= *de*) and *P. thunbergiana* (= *th*). A total of 58 compounds was identified.

Trispecies hybrids showed the same qualitative pattern of the needle volatile composition as their parents. However, there were quantitative differences in several major terpenes between them. The hybrids *nisyde* were more similar to the female parent (F_1 hybrid *nisy*), whereas the hybrids *nisyth* were more similar to the male parent (*P. thunbergiana*). The intermediary quality of the trispecies hybrids for most components in relation to their parents, confirms their hybrid character.

P2047. Anti-Trypanosomal secondary metabolites from two Cameroonian medicinal plants

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In the search of our course for antitrypanosomal compounds from natural source, two plants used in central and western part of Cameroon to treat several diseases were investigated: *Xymalos monospora* Baill (Monimiaceae) and *Millettia griffoniana* Bail (Leguminosae). From the stem bark of *Xymalos monospora*, five alkaloids including the new *Epi*-temuconine were isolated. From the seeds of *Millettia griffoniana*, twelve isoflavonoids with five new namely 7-methoxyebenosin, griffonianone (E-H) were isolated. The structure of compounds were established by MS, 1D and 2D spectroscopy including DEPT, COSY, HMQC and HMBC experiments. Crude extracts as well as pure compounds have been tested for growth inhibitory activity in vitro vs. Bloodstream forms of African trypanosomes. IC₅₀ values in the range of 1-3 µg/ml were found for many of pure compounds isolated.

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P2048. Modulation of GABAergic Neurotransmission by a New Component from *Cyperus Rotundus*D. U. Lee¹, B. S. Koo², S. I. Lee³;¹Department of Biotechnology, Dongguk University at Gyeongju, Gyeongju, Republic of Korea, ²Department of Oriental Neuropsychiatry, Dongguk University, Seoul, Republic of Korea, ³School of Life and Health, Sorabol College, Gyeongju, Republic of Korea.

We report the isolation of some components, including new sesquiterpenes, from the active fraction of *Cyperus Rhizoma* and the agonistic property of a newly identified constituent from this plant on the rat cerebrocortical benzodiazepine receptor. Four sesquiterpenes, -selinene, isocurcumenol, nootkatone and aristolone and one triterpene, oleanolic acid were isolated from the ethylacetate fraction of the rhizomes of *Cyperus rotundus* and tested for their ability to modulate γ -aminobutyric acid (GABA_A)-benzodiazepine receptor function by radioligand binding assays using rat cerebrocortical membranes. Among these compounds, only isocurcumenol, one of the newly identified constituents of this plant, was found to inhibit [³H]Ro15-1788 binding and enhance [³H]flunitrazepam binding in the presence of GABA. These results suggest that isocurcumenol may serve as a benzodiazepine receptor agonist and allosterically modulate GABAergic neurotransmission via enhancement of endogenous receptor ligand binding.

P2049. Isolation and Identification of the Major Terpene Components of the Essential Oil of *Salvia officinalis* L. (Sage) Grown in Egypt

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This paper deals with the isolation and identification of the major terpene constituents of the essential oil of *Salvia officinalis* (commonly known as Sage), a herb that long been used to impart aroma and flavor to food. The essential oil extracted from the leaves is important due to its application in traditional medicine and its properties as antibacterial, antiviral and cytotoxic properties. The essential oil was isolated from the leaves of *Salvia officinalis* by steam distillation using a Clevenger-type apparatus. Identification of the terpenes constitution of the essential oil was carried out using gas chromatography equipped with flame ionization detector, as well as gas chromatography-mass spectroscopy. From the analysis data it was clear that the major terpene components constituting the essential oil are: -thujone 30.21%; camphor 20.5%; -thujone 11.34% and 1,8-cineol 9.21%. These values were compared with a previous reported data observed from *Salvia officinalis* essential oils grown in other different environments around the globe.

P2050. X-ray structure of a blue complex pigment from the blue flowers of *Centaurea cyanus*M. Shiono¹, N. Matsugaki², K. Takeda³;¹Department of Physics, Graduate School of Science, Kyusyu University, Hakozaki 6-10-1, Fukuoka 812-8581, Japan, ²Photon Factory, High Energy Accelerator Research Organization, Oho 1-1, Tsukuba 305-0801, Japan, ³Department of Biology, Tokyo Gakugei University, Koganei, Tokyo 184-8501, Japan.

The blue pigment of the cornflower, named protocyanin, has long been investigated, but its precise structure has remained unclear. Our recent research demonstrated the components of protocyanin to be anthocyanin (AN), flavone glycoside (FL), Fe³⁺, Mg²⁺ and Ca²⁺ ions and we succeeded in the reconstruction of protocyanin. In this study, we revealed the X-ray structure of protocyanin. The crystal structure of the reconstructed protocyanin was determined at a resolution of 1.05 Å. The refined molecule has pseudo three-fold symmetry and four metal ions, Fe³⁺, Mg²⁺ and two Ca²⁺, align along the pseudo three-fold axis. The four metals are coordinated to six AN molecules and six FL molecules. The inner Fe³⁺ and Mg²⁺ ions are each coordinated to three AN molecules respectively, while the outer two Ca²⁺ ions are each coordinated to three FL molecules. Both AN and FL molecules are self-associated with each other as AN-AN and FL-FL in pair and this hydrophobic association also exists between AN and FL molecules. Protocyanin is a tetra-metal (Fe³⁺, Mg²⁺, 2Ca²⁺) nuclear complex of twelve molecules of anthocyanin and flavone glycoside, a new type of supramolecular pigment.

P2051. Curcuminoid Fingerprint of *Curcuma longa* Linn.L. Sriphong¹, P. Yaipakdee¹, U. Sotanaphun²;¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Nakorn Prathom, Thailand, ²Department of Pharmacognosy, Faculty of Pharmacy, Silpakorn University, Nakorn Prathom, Thailand.

Curcuma longa Linn. (family Zingiberaceae), known as turmeric has been widely used as a natural food coloring agent and major component of curry powder as well as an anti-inflammatory agent

and the treatment of flatulence, dyspepsia, hepatitis, jaundice, menstrual difficulties and many other disorders. In Thailand this medicinal plant has been used as herbal drug and herbal drug preparations. Since chemical fingerprint shows an important mean for quality control of herbal medicines. Curcuminoid fingerprint was established using thin layer chromatographic method for determination of three major curcuminoids; curcumin, desmethoxycurcumin and bisdesmethoxycurcumin. The chromatographic chemical profiling method demonstrated successful applications to identify and assess the consistency of turmeric from different sources. This curcuminoid fingerprint represented the characteristic components that would be useful for the quality control and chemotaxonomic determination of turmeric.

P2052. Qualitative and Quantitative Radiation Protection Analysis of Mucosa of ICR Strained Mice Using Selected Herbal Extracts such as GC-2112 from Garlic (*Allium sativum*) and GX-2137 from Ginseng (*Panax sp.*)

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Earlier reports showed that ginseng has significant radioprotective and stimulatory effect on the recovery of the lymphocytes and leukocytes

Using graded absorbed doses of radiation (1.5, 5, 20, 50 Gy) applied in ICR strain male white mice which was injected with GX-2137 from ginseng (*Panax sp.*) and GC-2112 from garlic (*Allium sativum*) was tested to prove some radioprotective efficiency. The herbal extracts were injected intraperitoneally and the experimental mice were sacrificed 2 and 48 hrs. post-irradiation. Factors such as analyzing kinetics of critical tissue parameters (length of villi, the number of crypt and villi cells and cell density) and determining the Relative Protection Efficiencies (RPE) using quantitative histopathological techniques were used to quantify the radiation protection assay in the duodenum of ICR strain mice.

Results showed that GC-2112 and GX-2137 protected the villi structures. After 2 hrs. post irradiation, tissue degeneration was evident. RPE values of significant radioprotection of the crypts is demonstrated at absorbed dose. It was found that some villi cells are even viable at non-physiologic dose of 50 Gy.

P2053. Chemical composition and antibacterial activity of essential oil of *prangos ferulacea L.*

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South-West of Iran which is mainly used as rich herb in animal feeding. This plant was collected from Broujerd mountains of Lorestan province in Iran. The air-dried epigeous parts were subjected to *hydrodistillation* method (in a yield 0.45% W/W). Identification of essential oil constituents was made by GC and GC/MS. The antibacterial tests were carried out by drilling well and measuring diameter of inhibition zone.

A large proportion of the oil was composed of hydrocarbon monoterpenes. The major component is α -pinene (36.6%) and β -pinene (31.1%). The results of the antibacterial activity showed that the oil was active against most of the positive and negative bacteria specially *Staphylococcus aureus*.

P2054. Isolation of curcumin from curcuma amada rhizome and studying the effects of penetration enhancer and vehicle type on the percutaneous permeation of curcumin and its total extract rhizom

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Curcumin is the principle pigment and active component present within the rhizome of various species of curcuma genus. It has several pharmacological effects, such as: anticancer, antioxidant, antihepatotoxicity and antiinflammatory activities. In this study attempts were made to evaluate the extent of percutaneous permeation of curcumin for topical application as well as its isolation from curcuma amada rhizome. To perform this study, curcumin was isolated from *C. amada* rhizome. Then four semisolid topical formulations of curcumin in the form of gel, cream

and ointment were prepared and the effects of these vehicles on the permeation of curcumin through rat skin, using the static diffusion cells, were studied. Results of these studies showed that gel is the best vehicle for topical formulation of curcumin. Finally, the permeation of hydroalcoholic mixtures of curcumin and *C. amada* rhizome total extract, through rat skin was also studied in the presence or absence of penetration enhancers, such as aqueous mixture of lecithin (1 mg/mL), aqueous solution of sodium lauryl sulfate and mixture of ethyl acetate: n-hexane (1:1, V/V). Results showed that the absorption rate of curcumin and *C. amada* total extract through rat skin were increased by ethyl acetate: n-hexane about 2.23 and 1.50 times, respectively.

P2055. Distribution and Accumulation of Ginsenosides in *Panax quinquefolium* during Plant Growth

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Ginsenosides are the secondary metabolic products and active components in *Panax quinquefolium* that exert medicinal and nutritional effects on human beings. The research work determined the concentrations of ten ginsenosides in the leaves, stems, root cortex, phloem and xylem in four-year-old American ginseng at nine growing stages of sprout, separation of leaf and stem, flowering, green fruit, red fruit, fruit abscised, yellow leaf, leaf abscised and plant dormancy from early spring to late autumn. The results demonstrated that the highest concentrations of ginsenosides in stems, leaves, root cortex, phloem and xylem showed at the growing stages of flowering, red fruit, flowering and fruit abscised, fruit abscised and leaves abscised, respectively. The sequence of the accumulation of ginsenosides in American ginseng seems from the stem and leaves to root cortex, phloem and xylem. The main individual ginsenosides in the leaf and stem were Re and mRd, while in root were Re, mRb₁ and Rb₁. From the root cortex to phloem and xylem, the percentage of Re in total ginsenosides showed decrease, while the percentages of mRb₁ and Rb₁ in total ginsenosides were increase.

P2056. Flow cytometric investigations with an arabinogalactan-protein (AGP) from *Echinacea purpurea* and human leucocytes

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An AGP from pressed juice of the aerial parts of *Echinacea purpurea* is a high molecular weight glycoprotein (1.2x10⁶ Da) with a highly branched carbohydrate moiety of >90% (w/w), mainly consisting of 3-, 6- and 3,6-linked β -D-Galp residues, substituted with terminal -L-Araf residues and terminal GlcA and has been shown to have complement-stimulating activities *in vitro* (1, 2). In order to get information about possible interactions of AGP with the immune system on a cellular level, we generated polyclonal antibodies against this AGP. Araf residues form a key part of the antigen epitope for these polyclonal antibodies (3). Flow cytometric investigations show binding of the AGP to the cell surface of human leucocytes (lymphocytes, monocytes and granulocytes). Competition assays with two antibodies directed against CD4 and CD8 revealed no interaction of AGP with these receptors, leading to the conclusion that binding of AGP is mediated via different structures (4).

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P2057. Volatile Composition and in Vitro Antimicrobial and Antioxidant Activities of the Essential Oil of *Laurus nobilis L.* Leaves from Turkey

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In the present study, essential oil from the leaves of laurel (*Laurus nobilis L.*, Lauraceae) grown at 1100 m altitude in Turkish state forests (Andirin district), was obtained by steam distillation. The volatile composition of essential oil was analysed by GC and GC-MS. It contains 54,7 % 1,8-cineol, 25,1 % monoterpene hydrocarbons, 7,8 % monoterpene alcohols and carbonyls, 9,3 % monoterpene acetates and 0,95 % sesquiterpene hydrocarbons

as well as 0,27 % sesquiterpene alcohols and oxides and 0,33 % eugenol derivatives. The reducing power, antioxidant and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities of the essential oil were found to be low, compared to ascorbic acid, and BHT, respectively. Antimicrobial activities of the essential oil of the leaves of *L. nobilis* were determined using agar-disc diffusion method. Laurel essential oil shows good antimicrobial activities against *Escherichia coli*, compared to control antibiotics. No activity could be measured with *Yersinia enterocolitica*, *Micrococcus luteus* and the yeast *Kluyveromyces fragilis*.

P2058. Utilization of *Wolffia Arrhiza* extracts for protein rich husk and seeds yield of lentil and its socio-economic value

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Wolffia arrhiza is known as noxious weed and rated as one of the ten major weeds growing in South-East Asia, commonly infesting natural impounded waters in India. Studies on effect of *W. arrhiza* extract on protein rich husk and seeds yield of lentil (*Lens culinaris*, variety D.P.L.-15) were made. Pre-soaking seed treatments for 12 and 24 hrs. were given with 1, 2 and 5 percent ether and water extracts of *W. arrhiza*. Synthesis of protein and consequently protein contents were increased in husk and seeds yield of lentil produced to the maximum extent in treatments 12 and 24 hrs. with 5 per cent ether and water extracts. Results are statistically significance at 5 percent error probability.

The NSSO reports showed that 42 percent of the rural and 49 percent of the urban population receive less than accepted norms of daily calorie intake (2200K Cal). The average intake of pulses is around 37 g. per capita per day as against the recommended dietary allowance of 50g. Too little energy and too little protein in the diet of about 80 million children have caused several disorders associated with protein energy malnutrition (PEM).

Present finding are of both social and economical significance of malnutrition in children, human beings, cattle and need practical adoption in agriculture of lentil by growers of commercial crop.

P2059. Volatile Composition and In Vitro Antimicrobial and Antioxidant Activities of the Essential Oil of *Laurus nobilis* L. Leaves from Turkey

D. Metin;

KSU, Kahramanmara , Turkey.

In the present study, essential oil from the leaves of laurel (*Laurus nobilis* L., Lauraceae) grown at 1100 m altitude in Turkish state forests (Andirin district), was obtained by steam distillation. The volatile composition of essential oil was analysed by GC and GC-MS. It contains 54,7 % 1,8-cineol, 25,1 % monoterpene hydrocarbones, 7,8 % monoterpene alcohols and carbonyls, 9,3 % monoterpene acetates and 0,95 % sesquiterpene hydrocarbones as well as 0,27 % sesquiterpene alcohols and oxides and 0,33 % eugenol derivatives. The reducing power, antioxidant and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities of the essential oil were found to be low, compared to ascorbic acid, and BHT, respectively. Antimicrobial activities of the essential oil of the leaves of *L. nobilis* were determined using agar-disc diffusion method. Laurel essential oil shows good antimicrobial activities against *Escherichia coli*, compared to control antibiotics. No activity could be measured with *Yersinia enterocolitica*, *Micrococcus luteus* and the yeast *Kluyveromyces fragilis*.

P2060. A data bank of terpene species of Italian Flora

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Occurrence and distribution of plant volatiles has been significantly known in the last 15 and permits preliminary overviews. We taken into account Italian flora for the availability of a detailed flora and occurrence of different biomes. We put in databank and analysed relation between contents and emissions of terpenes and ecological characteristic of species; we taken in consideration isoprenoids and sesquiterpenes, constituents of essential oils and particularly active from the metabolic point of view. Furthermore isoprenoids are the principal constituents of bVOCs, important in pollution problems. We cross data relative to presence in plants with information related to taxonomy, corological type, biological forms, habitat, syntaxonomy to verify correspondence between emission and storing of terpenes and ecological characteristic of plants and to identify trends in relation to taxonomical or ecological

groups. Our statistical analyses can help the comprehension of their function in relation to environment and climatic condition.

P2061. Taiwan Biological Database and Its Use in Vegetation Classification

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Taiwan Biological Database was established in 1999 to hold data from Taiwan biological survey project. The project recorded sample site, species name, and other data the individual researcher needed, such as sample area size. The locality accuracy is required to be within 100 m, but in most cases the data are recorded within 10 m in accuracy. In the current Taiwan vegetation inventory and mapping program, because steep topography and more time required to record species in the releves from tropical and subtropical areas, the program time would not be able to survey every polygons delineated. As the result, the vegetation samples are taken from the delineated polygons with greater dissimilarity, and the polygons with Biological Database data in will not be surveyed to have more different-class polygons sampled. Data from Biological Database are combined with current and historic vegetation samples to construct a vegetation classification system. The classification systems with and without Biological Database are compared.

P2062. Vaccinio - Piceetea Br.-Bl. in Br.-Bl. et al. 1939 Class in Moldova (Romania)

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Moldova is one of the historical provinces of Romania, situated in the E-NE part of the country. The western limit of this province is represented by one branch of the Carpathian Mountains, namely the Oriental Carpathians. Forest phytocoenosis - made of coniferous forests - covers a large area in the Romanian Mountains. In Moldova there were identified 8 associations: *Hieracio transilvanici - Piceetum* Pawl. et Br.-Bl. 1939; *Soldanello montanae - Piceetum* Volk in Br.-Bl. et al. 1939; *Dicrano - Pinetum* Preissing et Knapp in Knapp ex Oberd. 1957; *Pino cembrae - Piceetum* Chifu et al. 1984; *Piceeto - Juniperetum sibiricae* Br.-Bl. 1930; *Rhododendro - Pinetum mugii* Borza 1959 em. Coldea 1985; *Leucanthemo waldsteinii - Piceetum* Krajina 1933; *Hieracio transilvanici - Abietetum* (Borhidi 1971) Coldea 1991. These associations are included in 5 alliances (*Piceion excelsae*, *Dicrano - Pinion*, *Pinion mugii*, *Chrysanthemo rotundifolii - Piceion*, *Abieti - Piceion*) and 2 orders (*Piceetalia excelsae*, *Athyrio - Piceetalia*).

P2063. Spatial patterns of plant communities in a protected semiarid region in tropical Mexico.

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Although the debate on communities as natural or artificial units is a matter of the past, the identification of plant associations is still of great practical relevance for the management of protected areas and for attempts to predict possible plant community shifts with anticipated climate or other environmental changes. We explored vegetation-environment relationships in the semiarid Zapotitlán Valley, a local basin in the heart of the Tehuacán-Cuicatlán Biosphere Reserve in tropical Mexico. We analyzed spatial patterns of plant communities from 27 sampling sites in 2003. Environmental variables were obtained from field surveys, remote sensing and cartographic sources. Ordination (Canonical Correlation) and classification (Cluster and Discriminant) analyses allowed the definition of six plant associations and the detection of major environmental gradients. These groups differ in absolute abundance and abundance ranking, but showed only slight differences in species composition. Discriminant functions were incorporated in a Geographic Information System to generate a cartographic model which, in turn, was used to produce a vegetation map.

P2064. Evaluation of classical and numerical approach in syntaxonomy

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Striving to establish generally acceptable principles in syntaxonomic researches, methods of numerical ecology are today often used.

In contrast to classical approach bringing along high level of subjectivity, methods of numerical ecology having an opportunities for needed sciences objectivity.

However, methods of numerical ecology could not play the basic and only way in establishing of syntaxonomical units, as well as characteristic species of particular communities.

Traying to accomplish the higher level of objectivity, methods of numerical ecology are using a numbers of relevés with different origin. It is not possible to avoid the fact of different authors subjectivity level. Using relevés with different origin is establishing of assembly, but not a system as suitable base.

No one computer program is not able to assess importance of particular species. Programs are registering presence, absence and quantitative aspects of all species equally.

Next step in progress of objectivization could be combining classical and numerical syntaxonomy through previous ranging of species by its phytocoenological belonging.

P2065. The Diversity of Vegetation of Serbia and their Relationship to European Habitat Classification

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In co-production with Ministry for Sciences and Nature Protection of Republic of Serbia and Faculty of Biology University of Belgrade, group of scientists have compiled a list of phytocoenological names (from classes to subassociations and facies) in their overview covering the whole of Serbia.

Initial Vegetation of Serbia Data Base was made. Position of territory of Serbia between several phyto-geographical regions C. European, Pontic-S. Siberian, Mediterranean and Alpine generates great floristic and vegetation diversity. Based on checking of 1.580 phytocoenological references, 2.160 names of associations, subassociations and facies was classified into 40 classes, 85 orders, 184 alliances and 21 suballiances.

The all associations and subassociations with their facies are cross-referenced with correspondign EUNIS, PALEARCTIC, CORINE, RAMSAR, EMERALD and NATURA habitat types.

P2066. Classifying the main plant communities in the reserve "Kamenshtitsa", Central Stara planina mountain

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The aim of the present investigation is classifying the vegetation in the reserve "Kamenshtitsa", Central Stara planina mountain.

The research was worked out during the vegetation periods in 2002 and 2003 year. For implementation of the aim the following tasks have been set: identification of the main plant communities in the reserve and an attempt of composing a geobotanical map of the vegetation. For solving these tasks the principles of the two phytocoenological schools are used - the Braun-Blanquet and the Russian school. During the terrain work over 45 phytocoenological descriptions were made. The described associations are of mixed origin and belong to several main vegetation formations: that of *Fagus sylvatica*, *Quercus daleschampi*, *Festuca dalmatica* and others. A map of the plant associations is created. Some comparisons with similar investigations are made. The results are used for making conclusions for the succession changes in the reserve.

P2067. Leaf physiognomic communities: A new approach for vegetation classification

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A new approach of vegetation classification is presented which is based on leaf physiognomic characteristics of vegetation. Contrary to many existing vegetation classifications leaf physiognomic traits of vegetation are regarded as chronological independent classification units. This circumstance allows the application of the concept of "leaf physiognomic communities" to extant as well fossil vegetation types. For definition of the syn-physiognomic units 25

leaf physiognomic characters of 108 European hardwoods are recorded and scored. Based on synthetic chorologic floras a grid data set comprising the leaf physiognomic character composition of the vegetation was compiled. A hierarchical classification of the grid data using cluster analysis reveals 5 principal leaf physiognomic vegetation units in Europe (Mediterranean, oceanic, Scandinavian, sub-continental and continental class). Ordination methods (PCA, RDA) allow a further analysis of these classes in the leaf physiognomic character space. As an example two fossil leaf assemblages from the Paleogene of Spitsbergen and Switzerland demonstrate the application of the classification.

P2068. Classification of vegetation on exposed pond bottoms of the Czech Republic

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A classification of vegetation of the classes Isoëto-Nanojuncetea and Bidentetea by Cocktail method was performed. The method is based on the creation of the specific groups of species under a supervision of an expert; the process is statistically controlled. An individual community is characterised by the presence of the specific species group or by the combination of more species groups, appropriately by their absence. The communities which are lacking such a group of species, can be defined by dominants in some cases. The "dominant approach" had to be used in most Bidentetea communities, because using the "species group approach" the Bidentetea are not clearly defined versus Isoëto-Nanojuncetea communities. The results of the Cocktail method were compared with the results of cluster analysis and with the definition of individual associations of Isoëto-Nanojuncetea and Bidentetea, traditionally accepted in Czech phytosociological literature and based on methods of "classical" phytosociology. The Cocktail classification lead to the broadly-defined plant communities and to the reduction of a number of described association.

P2069. Formalized classification of ombrotrophic bog vegetation of the Czech Republic

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The vegetation of Czech ombrotrophic bogs and allied bog woodlands has been traditionally classified into 11 associations of the class *Oxycocco-Sphagnetum*, 6 as. of the class *Vaccinio-Picetea* and 3 as. of the class *Scheuchzerio-Caricetea fuscae*. Particular associations are conventionally defined in the different ways, mostly by species composition and dominance, but also by habitat and geographical characteristics. We used the procedure of formalized reproduction of an expert-based vegetation classification to define the associations in a formal way. Applying the Cocktail method to a geographically stratified data set of 21,795 relevés of all Czech vegetation types, we defined groups of species with the statistical tendency of joint occurrences in vegetation. Combinations of these species groups by logical operators and dominance of some species yielded formal definitions of associations. We rejected the associations that could not be reproduced by any combination of species groups or species dominance and the associations lacking diagnostic species that could be derived from entire data set by calculating fidelity of species to the associations.

P2070. Determination of plant ecological groups in the beech(*Fagus orientalis* Lipsky)forests, Siyahkle, eastern Guilan, Iran

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The aim of this research was to determine plant ecological groups and their relations with topographical factors in the beech forests of Shenrood, district No.6, Siyahkle. The systematic random method and nested plot sampling procedure were used for data

collection. Within each sampling plot tree, shrub, herb and tree seedling layers were collected and identified, in spring and summer. Their abundance- dominance was estimated based on Braun-Blanquet criterion. Ecological groups were diagnosed by means of TWINSPLAN classification method. The results showed that three ecological groups were found in the tree and shrub layer. In the herbal and tree seedling layer of spring collection, four ecological groups and in the herbal and tree seedling layer of summer collection, three groups were found. Comparing distribution of plant ecological groups with aspect, slope and elevation revealed that between them and aspect, and also some ecological groups there were significance relationships between them and aspect and slope, while remarkable relationships were not observed between ecological groups and elevation.

P2071. Can plant community ordinations be affected by various sample plot sizes in grasslands?

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Vegetation samples from plots that are stored in phytosociological databases often differ by one or two orders of magnitude in size, even within a single vegetation type. This fact may influence vegetation analysis, but the extent of this possible influence has not been investigated so far. The effect of sample plot size on ordination results was investigated in grassland vegetation in southern Moravia (Czech Republic), using data from nested plots of 1, 4, 16, 25 and 49 m², placed along two ecological gradients: long gradient (including different vegetation types and various climatic condition in a larger area) and short gradient (small area with a floristically homogeneous vegetation type). In both gradients the smallest plot sizes showed the most deviating patterns in ordinations, while samples of larger sizes gave similar results. The transformation of percentage cover also affected the results, more so when samples were collected along short ecological gradient. Samples differing in size by one order (1 and 49 m²) seem to be inappropriate to combine together in ordination analysis, especially when they were sampled along a short ecological gradient.

P2072. A comparison of unsupervised and supervised classification of meadow vegetation of the Czech Republic

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A comparison of two different classifications of a large phytosociological data set of meadow vegetation of Molinio-Arrhenatheretea class from the Czech Republic is presented. The first classification was performed using cluster analysis and in the second classification vegetation units were defined by combination of species groups created by Cocktail method. While in cluster analysis vegetation plots are classified by an unsupervised algorithm of a computer program, Cocktail classification is basically created by expert knowledge. In cluster analysis, a resemblance measure and a group linkage method are chosen and then we cannot influence the process of classification and assignment of relevés to the groups. By contrast, in Cocktail classification an expert makes subjective choices during the classification process while the program suggests possible solutions and ensures that particular steps in the classification process are applied consistently throughout the data set.

P2073. Can be plant species groups transferred across scales?

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Three datasets were sampled: a floristic one on a 0.5 km² grid, another floristic one on a 0.13 km² grid, and a vegetation dataset at the plot scale (0.200 m²). Species groups (SG) were extracted from all the datasets using the method Cocktail and the SG were assigned crosswise from one dataset to the other two ones. Correlation between GIS-variables and species distribution at each scale was calculated by multivariate analysis. The SG that were common to all datasets (across all scales) represented the main

ecological gradients of the area. SG derived from broader scale data had generally a lower correspondence when applied to fine-scale data than *vice versa*. Some SG were less scale-dependent (i.e. spatially stable) than others. The most important variables for the spatial distribution of species was the size of forest area, whilst most variability of data explained the fine-scale. A possible application is to predict the potential occurrence of missing species in areas with incomplete floristic or vegetation surveys.

P2074. Classification of boreal forest vegetation in the Middle and Eastern Europe: a comparison

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Boreal forests in European Russia are divided in four zones: north, middle, south and hemiboreal. Zonal associations differentiate every zone. Besides there are plant associations that occupy different habitats in every zone. Boreal forests in Eastern Europe are very homogeneous from west to east and it is impossible to distinguish any geographical vicariants on the level of association or subassociation.

Comparison of classification in Middle and Eastern Europe is very difficult task. Common classifications of boreal forests are absent in these regions. Diagnoses of associations are not formulate explicit. Characteristic and differential species are not constant and have geographical and ecological ranges that do not coincide completely with range of association. Only Calamagrostio arundinaceae-Piceetum and Sphagno girgensohnii-Piceetum from north-eastern Poland correspond partly to associations of spruce forests in Russia.

P2075. The "total cover value ratio" - an alternative fidelity measure for the determination of diagnostic species

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The fact that species differ in their diagnostic value is reflected by the concept of fidelity. Recently, several fidelity measures have been proposed, based solely on constancy. BERGMEIER et al. and DENGLER proposed a threshold for diagnostic species which seems rather weak for a sharp delimitation of associations. Moreover, constancy is strongly dependent on the size of the relevés used, making it impossible to compare e.g. forests with herb vegetation. An alternative solution was presented by BARKMAN who proposed the "total cover value" (TC), defined as the average cover value of a species in a syntaxon, also taking into account the relevés where it is absent. Here, a more general definition is presented which takes into account also the size of each relevé. In this form, the TC is no longer dependent on size or shape of the relevés used. Fidelity can be defined by the TC ratio which is the TC of a species in a given syntaxon divided by its TC in the compared syntaxon. Threshold values for diagnostic species are suggested, based on the assumption that a good diagnostic species should (almost) be restricted to the syntaxon for which it is diagnostic.

P2076. Stratified resampling of vegetation plot databases as a bias reduction technique in classification studies

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Large vegetation plot databases are increasingly used for description of vegetation patterns over large areas and testing various ecological hypotheses. The analyses, however, may be seriously biased due to heterogeneous origin of the data in such databases, where many records are based on opportunistic sampling. We propose some resampling strategies that may reduce such bias and compare classifications of the data sets that were prepared with these resampling strategies. As a demonstration database we used 6050 plot records of meadows and mesic pastures from the Czech Republic. We used the following resampling strategies: (1) Geographical stratification; Three types of habitat stratification: (2) by phytosociological associations; (3) by numerical classification; (4) by Ellenberg indicator values; (5) selection of plots with potential diagnostic species. We also used four combinations of the geographical stratification with the other methods. The results indicate that stratified resampling may strongly influence the results of classification and possibly reduce the bias inherent to large databases.

P2077. Intrapopulation variability of *Abies alba* from the Tisovik Reserve expressed in needle traitsE. Pawlaczyk¹, M. A. Bobowicz¹, A. F. Korczyk²;¹Department of Genetics, Adam Mickiewicz University, Poznan, Poland, ²Natural Forest Department, Forest Research Institute, Bialowieza, Poland.

European silver fir (*Abies alba* Mill.) is a mountain tree, but sometimes it may occupy lowlands, especially in northern parts of its distribution. The geographical distribution of silver fir is limited mainly to the Central-European mountains and in Central Poland reaches the northern border. However, 120 km on the north from the border of range silver fir occurs in small, natural and isolated populations in Bialowieza Primeval Forest - the Tisovik Reserve (Byelorussia).

The human activities caused the severe losses in this stand thus the preservation of gene pool should be entered upon at an urgent mode. Bearing this in mind, our investigation aimed at describing the intrapopulation structure and genetic variability of silver fir from the Tisovik Reserve.

One-year old needles were collected from silver fir trees and characterised in respect to 12 morphological and anatomical traits and next were subjected to multivariate statistical analyses. Studied trees within the population showed a homogeneous character so it may be concluded that individuals from the Tisovik Reserve showed the uniform character and this population is weakly differentiated.

P2078. Tree diversity and regeneration dynamics of a montane forest in Ecuador and factors affecting itJ. Homeier¹, S. W. Breckle²;¹Albrecht-von-Haller-Institute for Plant Science, Göttingen, Germany,²Department of Ecology, Bielefeld University, Bielefeld, Germany.

Field research for this study was carried out on the eastern slope of the Cordillera El Consuelo in Southern Ecuador. On permanent plots of 400 m² situated in altitudes between 1850 and 2450 m above sea level all trees with a DBH (diameter at breast height) of 5 cm or more were inventoried. Woody plant regeneration was investigated. With ordination methods based on tree species composition in the plots forest types could be distinguished. These types are not only distinct in species composition, but also in diversity, structural features like tree basal area and radial growth rates. Structure, species composition and regeneration dynamics are closely related to topography. Highest species diversity was found on lower slopes and in ravines, together with highest basal area and highest growth rates. Nutrient supply seems to be one controlling factor for tree diversity and growth. Specific site conditions influence growth and mortality of the woody regeneration.

P2079. Tall herb spruce-fir forests as a refuge for boreal forest plant diversityM. V. Bobrovsky¹, L. G. Khanina², O. V. Smirnova³;¹Institute of PhysicoChemical and Biological Problems in Soil Science of RAS, Pushchino Moscow region, Russian Federation, ²Institute of Mathematical Problems in Biology of RAS, Pushchino Moscow region, Russian Federation, ³Center of Problems of Ecology and Productivity of Forests of RAS, Moscow, Russian Federation.

We studied vegetation and soil in old-growth spruce-fir forests in North-East of European Russia (average age of spruce about 150 yrs, max. age - 380 yrs). According to ground vegetation composition we revealed 7 different forest types, and for each of them we estimated plant and soil diversity. The highest plant diversity (more than 40 vascular plants per 100 m², and 261 species from total 280 that we registered) correlated with the richest soil and was observed in tall herb spruce-fir communities (TH). The last were found in river valleys as well as at watersheds. Indicator species calculated by the IndVal method were: *Filipendula ulmaria*, *Cacalia hastata*, *Urtica dioica* etc. and *Aconitum septentrionale*, *Geranium albiflorum*, *Cirsium heterophyllum* etc. respectively. Then we noticed: tree fire scorches were absent in TH, and carbons in soil were met very rarely and deeply, while in other forests fire traces were met everywhere. We concluded: i) the appearance of tall herbs in spruce-fir forests is possible only under long-term fire absence; ii) TH are boreal forest refugium where the most of boreal species are stored; iii) TH are the most close to climax boreal forests.

P2080. Variation of Composition, Diversity and Structure of Vegetation along an altitudinal gradient in a Tropical Mountain Forest (SFF Guantá - Alto del Río Fonce, Colombia)

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There are two main hypotheses about how change the composition of vegetation in altitudinal gradients: the monotonic and the hump models. We studied the vegetation of a tropical mountain forest at the Guantá - Alto del Río Fonce natural park, on a gradient between 2000 and 3000 m of altitude. We established 5 stations for sampling, separated one another 250 m of altitude. Each station had 4 transects (50x5m), in which we sampled woody plants with dbh \geq 2.5 cm. We found 250 species. The richest families were Melastomataceae (21), Rubiaceae (19) and Araliaceae (10). Richness and diversity show significant differences between stations ($\alpha=0.05$, $p=0.014$ and 0.025) and they decrease with altitude. Regarding structure, we got significant differences in the total height and competition factor ($\alpha=0.05$, $p=0.004$ and 0.0025), but they did not follow any predictable pattern. Density, evenness, mixture coefficient and basal area did not vary significantly. The higher values of IVI increase conspicuously with altitude. We conclude that composition and diversity follow monotonic model. In contrast, we think changes in structure do not respond to altitude but to disturbances.

P2081. The fragmented Brazilian *Mata Atlântica* and Edge Effects: the case of *Reserva Biológica União* (Rio de Janeiro, Brazil)P. Pena Rodrigues^{1,2}, M. T. Nascimento², J. A. Braga¹, M. A.Iguatemy¹, R. C. R. Abreu¹;¹Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil,²Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Brazil.

The *Mata Atlântica* is severely threatened by habitat loss and fragmentation and remnants are still subjects to edge effects. Functional and structural alterations can degrade the fragments and leading this biome, still largely unknown, to extinction. This long-term multidisciplinary study initially aimed to investigate arboreal structure, floristic and local edge effects on trees and seedlings communities at *Reserva Biológica União*. Permanent plots nearly two deforested corridors, *Rede Elétrica* and *Gasoduto* and preserved *Interiors* were used since 1999. Trees communities were very rich (208 sp/ha) and diverse ($H'=4.9$ nits). Local structural patterns were altered by edge effects. Edges heterogeneity was observed in practically all analyzed parameters. Typically pioneers and the alien jack-fruit were edges exclusives. Seedlings showed low density and high mortality mainly on edges. The heterogeneity observed suggests that, if cease the anthropogenic impacts, as the edge effects observed, ecosystem can recovery natural patterns and process.

P2082. Edge Effects on *Mata Atlântica* seedlingsM. A. Iguatemy¹, P. Pena Rodrigues^{1,2}, M. T. Nascimento²;¹Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil,²Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Brazil.

Among the impacts that *Mata Atlântica* biome remnants are subject we highlight the processes generated by edge effects that potentially may lead to a complete degradation. This study includes a long-term perspective that aimed to investigate aspects of edge effects on seedlings communities' dynamics at *Reserva Biológica União* (Rio de Janeiro, Brazil), one of the newest Brazilian protected areas. Inside a multidisciplinary project permanent plot near two deforested corridors, *Rede Elétrica* and *Gasoduto* and preserved *Interiors* were used since 2000 to 2004 year. Comparisons were made and we found that smaller individuals were more susceptible to edge effects. Size structures and densities vary among the years, but clearly edges always were more heterogeneous (based on coefficients of variation) than interiors. Patterns observed indicate that edge effects are altered seedlings communities' properties and artificial limits maintenance may degrade the forest remnant. Otherwise buffer zones probably may avoid direct edge effects on seedlings.

P2083. Modelling of ground vegetation dynamics in forest ecosystemsL. G. Khanina¹, M. V. Bobrovsky², A. S. Komarov², A. V. Mikhajlov²;¹Institute of Mathematical Problems in Biology of RAS, Pushchino

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The method of predicting forest ground vegetation dynamics on a base of standard forest inventory data is proposed. We use the functional plant species groups, which have been described earlier. We found a distribution of the functional groups accordingly to tree dominant, soil fertility and moisture for Central Russian forests. The table allows for prediction of the dominant group in ground vegetation using forest inventory data. We applied model of forest growth EFIMOD to simulate dynamics of 'trees-soil' system at the stand level and proposed rules for predicting the group modifications at the simulated changes of tree species composition, dead wood biomass, soil organic matter and nitrogen dynamics. We estimated level of plant species richness for the forest type on the base of combining of dominant group in ground vegetation with tree dominant using database FORUS, which contains 9000 relevés from European Russia. The method was applied for a forest area under different forest management strategies.

P2084. Tree invasions into Seychelles tropical forests: ecophysiology of native vs. invasive species.

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For oceanic islands it is hypothesized that invasive plants possess new functional attributes that were not previously present in the native flora. The forests on the Seychelles granitic islands harbor one of the oldest and most isolated floras of the world that are ideal for comparing functional traits of native vs. introduced and invasive trees.

Growth characteristics of juveniles were studied in five native and five invasive woody plant species in field and common garden experiments. Light and nutrient availability were varied as main factors. In a water stress experiment seedlings were grown under different light and water availability.

Overall, the invasives showed higher growth rates and higher phenotypic plasticity than the natives by better exploiting high resource levels (light, nutrient). However, the two groups did not differ in their response to water stress. Generally, among-species variation in the responses to different resource levels and water stress were large.

In Seychelles, natural gap dynamics may provide sufficient opportunity for plastic invaders to spread because they survive in shade and rapidly exploit high resource levels in gaps.

P2085. Tree Diversity Patterns in Los Cedros de Pampa Hermosa, Chanchamayo, Junín, Perú.

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Tree abundance and species richness (dbh³10cm) was quantified in 1 hectare from the Cedros de Pampa Hermosa montane (CP) forest, one of the eight Pleistocenic refuges in the Eastern Andean rain forests. A total of 140 species, 72 genera and 36 families were recorded (~400 individuals), though species-area curves suggested that sample size underestimated local diversity. Lauraceae, Meliaceae and Moraceae were the dominant families, while tree species mostly contributing to total basal area were also of economical interest (*Cedrela lilloi*, *Guarea* sp. and *Guarea guidonia*). Indicator species showed that the study forest is little fragmented and locates in an ecotonal area between tropical rain and premontane forest. Tree community structure shown by multivariate ordination of 7 montane forest types in the Chanchamayo area (485 taxa), revealed the uniqueness of the CP forest. >50% of the taxa pool could not be keyed out to species level, so the effect of including/excluding morphospecies on community structure analysis was discussed. The features of the CP forest highlights the global importance of this site in conservation strategies focusing on montane forests.

P2086. Diversity and composition of dead wood inhabiting fungal and bryophyte assemblages in semi-natural beech forests in Europe

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Species composition and diversity of fungi and bryophyte assemblages occurring on dead beech trees were analysed in five European countries.

In the composition of fungi the effect of decay stage exceeded the effect of geographical difference, whereas for bryophytes it was the opposite. In the case of bryophytes in Slovenian hepatics, in Hungary and Denmark pleurocarpic mosses, and in The Netherlands and Belgium acrocarpic mosses were the most important.

The species richness of both groups differed among countries but their diversity patterns deviated. Slovenian sites were a biodiversity hotspot of bryophyte diversity (high species richness, presence of threatened species), Hungarian sites had an intermediate position while the Atlantic region had deteriorate assemblages. For fungi species richness was very high in Denmark, but the Hungarian and Slovenian sites were richer in threatened species. For both organism groups the most important factor in tree level species richness was the size of trees. For the diversity of both organism groups forest history, dead wood availability and continuity were more important than climatic conditions.

P2087. Dominant woody species near human disturbed landscapes on Mt. Makiling, Philippines

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Forty plots were set up near disturbed landscapes on Mt. Makiling, Philippines to know the woody species composition. A total of 92 species in 76 genera and 42 families were recorded. The agricultural farm and the residential had the lowest number of woody species (17-19). The forest edge had 25 species. The buffer zone and undisturbed forest had the highest number (45). Cluster analysis showed two distinct groups, the vulnerable and the more stable landscapes. At a lower dissimilarity level, the five land uses are distinct.

Basal area values indicate *Brassaia actinophylla*, *Cocos nucifera*, *Ficus* spp., *Gmelina arborea*, *Spathodea campanulata* and *Sweitenia macrophylla*, as dominant in vulnerable landscapes. *Alangium longiflorum*, *Chisocheton cumingianus*, *Litsea garciae*, *Ludekia bernardoi* and *Pisonia umbellifera* dominate the more stable landscapes while *Arenga pinnata*, *Celtis luzonica* and *Diplodiscus paniculatus* are the most dominant in both the vulnerable and the more stable landscapes.

P2088. Study on structure and plant species diversity in the box tree (*Buxus hyrcana* pojark) sites, eastern Guilan, Iran

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The aim of this study was to characterize structure and plant diversity in the box tree sites, Anjilbon and Radarposhteh, eastern Guilan. The tree, tree regeneration and herbaceous species were sampled by using random-systematic method. To study structure, number per hectare and height curves, and mean of diameter, number, and basal area per hectare of box tree were obtained. Plant diversity in three layers was calculated by using diversity indices. Results revealed that mean of number per hectare of box tree in Anjilbon was higher than Radarposhteh, while mean of diameter, height and basal area per hectare in Anjilbon were lower than Radarposhteh site. Tree diversity and evenness mean in Anjilbon were higher than Radarposhteh, whereas tree richness in Radarposhteh was higher than Anjilbon site. Also, mean of diversity, evenness and richness of tree regeneration in Anjilbon were higher than Radarposhteh site. The mean of diversity and richness of herbaceous layer in Anjilbon were lower than Radarposhteh, whereas evenness mean in Anjilbon was higher than Radarposhteh.

P2089. Species Diversity of Undergrowth Plants in Phu Poe Community Forest, Kalasin Province, Thailand.

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Abstract

Phu Poe community forest in Tumbon Naboan, Ampur Kammuang, Kalasin Province, Thailand, consists of compound dry dipterocarp forest with mixed deciduous forest. The main objective of this study is to compile a checklist of undergrowth plants and explore their ethnobotanical information. The belt transect method, 10 meters wide with 1,200 meters long from ground level to the top of the mountain, was applied for plant collection. There were all together 16 belt transects with an interval of about 250 meters each. The plant specimens were collected from July to October 2003. General morphology and habitat of the plant collected with their uses (if any) were documented. A total of 101 species (72 genera in 49 families) were enumerated with one species each of non-seeded plant and the gymnosperm, 20 species (11 genera in 7 families) of the monocotyledonae and 79 species (59 genera in 40 families) of the dicotyledonae. Family Zingiberaceae were the greatest in number (10 species) of all the specimens collected.

P2090. Floristic similarity among fir forests on the Trans-Mexican volcanic belt, Central Mexico.

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We studied the structure and floristic composition of a fir forest on the northern Sierra Nevada, Mexico based on sampling of 12 1000-m² plots for trees, 12 500-m² plots for shrubs, and 36 54-m² plots for herbaceous plants. The species structurally important were defined based on their relative importance value. In addition, we evaluated floristic similarity of this and other 12 fir forest sites located on the Trans-Mexican volcanic belt (TMVB). Floristic similarity was evaluated using classification and ordination techniques. The flora of these fir forests contains 76 families, 222 genera, and 510 species. A small percentage of the most common families contains a very high proportion of the genera and species. The differences in floristic composition among all sites are related with the geographical distance and elevation. A low proportion of species is common for all the fir forests studied which are important in defining forest structure at local and regional scales.

P2091. Laws of plant continuity

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Laws of plant continuity are formulated so:

1. Phytocenotic continuity is unique for every landscape, and continuity transitions are determined by species area covering.
2. The plant continuity in the landscape is determined by the climatic background.
3. Environmental-phytocenotic species ranges are changed according to certain standard, that is if several landscapes are ordinated according to background bioclimatic conditions improvement gradient, then environmental-phytocenotic species ranges and their phytocenotic optima will be displaced according to this gradient inside landscapes in the area of less favourable conditions.

These conclusions were made according to the examples of plantings in 4 landscapes. Three landscapes were investigated in the northern Transbaikalie, one landscape - in the Western Siberia. Types of communities for every landscape were ordinated into monocentric schemes according to: thermal factor, lythomorphic and hydromorphic. Then along the landscape gradient changes in environmental-phytocenotic species ranges were traced. 29 species of life forms and general for 3 or 4 landscapes were analyzed.

P2092. Species composition and stand structure of the beech forest in Taiwan

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Fagus hayatae Palib. ex Hayata is endemic to Taiwan. Although once more widespread during the Pleistocene, this species is now only limited to a few isolated fragments on ridgetops (1,300-2,000 m asl.) in northern Taiwan. The structure and species composition of the beech forest were studied on five isolated mountains. The beech usually forms pure stands on ridgetops and is mingled with broad-leaved forest at lower slopes and warm-temperate montane coniferous forest at the upper elevation limit. Comparison of TWINSPLAN groupings with the DCA ordination showed a definitive separation of communities along elevation and topographic gradients. The forest structure was characterized by a moderate density with an average basal area of 59 m²/ha. The beech contributed more than 70% of the total basal area. However, forest understory species varied considerably in their distribution and abundance among sites. Size-class distribution indicated a unimodal pattern reflecting that a much more drastic disturbance is needed for the recruitment of the beech forest.

P2093. Biologic forest ecosystem diversity and their impact in a semi arid land , analysis and followed by remote sensing "steppe in Algeria"

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Steppe presents a diversity floristique and faunique, and seen the unfavourable climatic conditions in the zone, one notes a deterioration of the physical environment notably the natural forest regression.

The use of images of the stage 195-36 of LANDSAT 5 in date of May 18, 1989. and of ALSAT1 2003 , at several dates of view holds has us permits to follow this regressive evolution and to make a report at the instant t of senalba forests and sahari.

The gait adopted in this project, at summer of treaty pictures satellites of LANDSAT and ALSAT1 of the different dates, that one permitted to make a thematic analysis of physical medium in steppe of the semi-arid floor permitting to determine the physical parameters of the middle (forest populations, vegetation steppe and soil) taking account of the human activity (reclamation, cut of wood and fire). This analysis has been followed of a specific treatment of data pictures, while using indications of vegetation and classifications supervised.

result is a cartographic support identifying the various components of physical medium and phenomenon of the deforestation.

P2094. Mycosociological and mycorrhizal estatus in the temperate rainforest of southern Chile under a low deposition climate

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The temperate rainforest of southern Chile represents an important reserve of extraordinary genetic, phytogeographic and ecological significance for the world. In addition, the chemistry of precipitations in southern Chile reflects one the closest approximations of pre-industrial atmospheric conditions.

Biotic components contribute to regulate the internal circulation and output of nutrients and these natural conditions give stability to the forest ecosystems.

We have done a mycosociological and taxonomical comparative study of the *Agaricales* that fructify in *Nothofagus* forests in the Central-Southern Chile and

the results show that the number of mycorrhizal fungi species varies between 40-50% and the saprophytic between 48 to 69%. A simultaneous analysis of the mycorrhizal status of the vascular flora of this forests was carried out, finding high proportions of plant species with arbuscular mycorrhizal symbiosis. Ectomycorrhizal plants associated exclusively with *Nothofagus* tree species.

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P2095. Structure of the forests in the Aragua River Basin, Municipio Piar, Monagas state, Venezuela.

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The area is located at the eastern section of The Coastal Cordillera Piedmont (10° 00' N, 63° 20' W, 320 - 580 m elevation).

Four parcels (250 x 20 m) were sampled, 10 cm DBH and smaller tree species were considered apart. Importance value as defined by Finol (1970) was calculated. Three main natural vegetation categories were found: 1. Riparian forest: 63 tree spp., 350 individuals/ ha, distributed in two layers, DBH average 23.97 cm, up to 20 m height, dominated by *Brownea capitella*, *Brossimum alicastrum*, *Siphoneugena dussii* and *Mouriri rhizophorifolia*; 2. Deciduous basimontane forest: 39 spp., 316 trees/ ha, which form two strata, DBH average 17.8 cm, up to 15 m height, dominated by *Bourreria cumanensis*, *Acacia paniculata*, *Pithecellobium unguicatii* and *Cordia alba*; 3. Transition savanna-deciduous forest: 34 spp., 292 trees /ha, DBH average 13 cm, up to 12 m height, dominated by *Copaifera officinalis*, *Guapira cuspidata*, *Randia armata* and *Erythroxylum impressum*. 179 species and 57 families were recorded. Mimosaceae, Fabaceae, Caesalpinaceae, Cappariaceae, Euphorbiaceae, Bignoniaceae, Flacourtiaceae, Myrtaceae and Rutaceae were the most diverse families.

P2096. Evaluation of South-Taiga Forest Biodiversity in north-east of Kostroma Region (European Russia)

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Forests of 90-160 yrs in south-taiga zone were inspected to reveal level of their biodiversity and its regulating factors. Zonal fir-spruce-dominated forests with lime (*Rhodobrya rosei*-*Piceetum abietis*) have been remained primarily in small-river valleys, whereas on watersheds there are birch-pine forests with spruce (*Dicrano-Pinetum*), but almost everywhere without fir and lime. Community ordination by NMS reveals not very close relations between species composition and catena position. In most of inspected catenas -diversity and -diversity of the forests are varying by contrast ways: species richness of communities and participation of ecological groups decrease along catenas upstairs, whereas -diversity (Whittaker index) increases. These trends can be explained as result of anthropogenic impact, especially periodical burning, on zonal watershed forests during 19-20th centuries. Interactions between burning and hydrogeological factors in catenas are discussed.

P2097. Plant species richness and diversity along an altitudinal gradient in the Sierra Nevada, Mexico.

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We studied plant species richness, and diversity associated with climatic and soil variables along a 1300 m elevation gradient on the northern Sierra Nevada in Mexico. Questions: does species richness and diversity vary significantly by elevation along the gradient? Are variations along the gradient monotonic, unimodal or otherwise? What are the main variables associated with these variations? Two 1000 m² plots for tree sampling, two 250 m² plots for shrubs and six 9 m² plots for herbaceous plants were placed at 21 sampling sites. The relationship between species richness and diversity and environmental variables was conducted by linear correlation and regression trees analysis (RTA). Species richness and diversity showed a unimodal pattern with high values in the lower half of the elevation gradient. Beta diversity increased steadily along the gradient, being lower between contiguous sites at intermediate elevations and nearly 100% between sites at the extremes of the gradient. The RTA suggests that differences in richness are mainly influenced by elevation and soil A₂ permanent wilting point, organic matter and horizon field capacity, and A₁ horizon Mg²⁺.

P2098. Floristic composition and regeneration of *Chamaecyparis*-dominated forests in northern Taiwan.

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The mid-elevation temperate forests of Taiwan support very diverse flora, and are especially rich in relict conifers. *Chamaecyparis formosensis* and *C. obtusa* var. *formosana* are the dominant species forming extensive forests. Floristic composition and regeneration patterns of these forests were studied in northern Taiwan. The results of DCA showed that significant altitudinal and

topographical gradients were present. *C. formosensis* often grows on damp ravine slopes and is intermixed with broad-leaved species at lower elevation. On the other hand, *C. obtusa* var. *formosana* usually forms pure stands interspersed with *Tsuga chinensis* var. *formosana* on higher slopes or ridges. Although natural disturbances play important roles in the regeneration of these two species, significant differences exist between them in regard to regeneration pattern. For both species abundant regeneration is dependent on large-scale disturbances such as erosions or landslides. However, coarse woody debris resulting from periodic windthrow provides additional habitat for the recruitment of *C. obtusa* var. *formosana*.

P2099. Floristic composition and plant communities at the Region of Los Loxicha, Sierra Madre del Sur, Oaxaca.

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Region of Los Loxicha, located at the Sierra Madre del Sur, Oaxaca, is characterized by high levels of ecological integration and plant cover. It exhibits high contrasts in its plant fisonomy, due to quick changes in altitude in a relative small area. Floristic composition of an altitudinal gradient was analyzed through systematic plant records, as well as by systematic samplings of four plant communities. We obtained 541 plant species belonging to 323 genus y 110 families. The most important family through the gradient was Leguminosae. Floristic composition is represented by tropical elements like *Oreopanax*, *Miconia*, *Piper*, *Ipomoea*, and *Begonia*, as well as boreal elements like *Cornus*, *Pinus*, *Prunus*, and *Quercus*.

Changes in species composition and abundance were detected through the altitudinal range of 1600 m. Some genus are found only in Subperennious forest (*Miconia*), or in Deciduous forest (*Swetenia*), while others, like *Quercus* or *Pinus* are found through the gradient, but with different densities. Plant species replacement indicates high levels of diversity and the effect of geological, climate and soil factors that generated complex microenvironments.

P2100. Climber abundance and diversity in African rain forests of the Albertine rift

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ABSTRACT. Climber abundance and diversity were assessed in twelve 1-ha (20 x 500-m) plots in four tropical high forests of the Albertine rift, western Uganda. A total of 5484 climbers (ramets) of diameter >1 cm representing 49 families, 111 genera, and 181 species was recorded. Average climber density was 457 individuals ha⁻¹. Climber densities were higher than recorded in Asian tropical forests but lower than recorded in forests of the Amazon. There were 24 climber families and 41 genera ha⁻¹. The most abundant families in number of stems were Celastraceae (23.5% of 5484), Apocynaceae (12.1%), Rubiaceae (8.2%), Piperaceae (6.4%) and Menispermaceae (5.7%). There were 51 species ha⁻¹ and the most abundant species was *Piper guineense* (Piperaceae) with 6.4% of the individuals and density of 29 ha⁻¹. Shannon's Diversity (H') ranged from 3.042 to 3.592. Climber species richness and diversity increased only slightly from forests in the south to those in the north. Based on quantitative plot data on climbers, the floras of Bwindi and Kasyoha-Kitomi are the most similar, followed by the floras of Kibale and Budongo.

P2101. Plant functional types, dispersion mode, pollination syndrome and spatial assemblage in an Amazon Forest Community, Brazil

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Functional groups could be based on plant reproductive strategies and assembly rules. Here we intend to test the hypothesis that species with similar reproductive and functional traits also have similar patterns of spatial distribution in an Amazon Forest community. A plot of 100 ha was completely surveyed (DBH> 30 cm) and trees plotted in an x and y system. ArcInfo 9.0 tools were used to calculate paired distances between individuals. A MANOVA was carried out to define a set of spatial variables:

nearest-maximum neighbor distance, mean, standard deviation. Cluster Analyses were performed to define Distance and Plant Functional Groups; MRPP, PCA and ordination were applied to test relationships among variables, functional types, reproductive mode and distance patterns. We found a significant interaction between seed size and distance between individuals ($F= 3.9$; $p<0.05$). However, animal and wind dispersed species presented similar patterns of assemblage. Although reproductive mode and spatial patterns generally were found to be distinct among functional types, other factors also contributed to the spatial patterns of community assemblage.

P2102. Validation of ecological-coenotical plant species groups in European Russian forests

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Ecological-coenotical plant species groups (ECG) are widely used in Russia to assess vegetation diversity and succession dynamics of forest ecosystems at different scales. We see ECG as a species assemblage formed under certain environmental conditions in a certain community type. We have proposed a joint expert-statistical approach to validate ECG systems obtained expertly. Ecological and coenotical species features for quantitative analysis were presented as variables of two types: (1) Ellenberg indicator values and (2) species scores derived from ordination of 2000 vegetation relevés. Discriminant analysis (DA) was used to identify misclassified species in a system of seven ECG. DA was run repeatedly on the whole set of variables and on various subsets of them with monitoring of results by experts on every step. The procedure continued until a position of every species in the ECG system fits both DA results and expert judgment. The approach allowed to greatly improve the ECG system, to put about 1000 vascular species into seven groups and to outline core and intermediate species.

P2103. Leaf anatomy of representative trees from the cerrado of São Paulo State, Brazil

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The Brazilian cerrado vegetation (savanna) is one of the most unique biomes that covers around 23% of the country and comprises a very diverse flora with more than 6000 species. However, despite its importance, modern agriculture has been developed in the cerrado areas and cattle are raised in enormous planted pastures, causing massive destruction. In order to identify any common and particular anatomical features of the cerrado's plants we studied the leaves of twenty of the more representative species of trees. In most species the leaves are hypostomatic, hairy and covered by a thick cuticle. The thickened walls of the epidermal cells are, in general, flattened at the outer periclinal side and convex at the inner periclinal side. The palisade parenchyma of the dorsoventral mesophyll occupies 50% or more of the lamina thickness and sclerified cells of different shapes are common in all representatives. All leaves have a dense and ramified vascular system. These features can be interpreted as adaptations to the environmental conditions in the cerrado areas, where high light intensities, low soil fertility, and a high frequency of fire predominate.

P2104. Variation in leaf morphology of *Quercus branti* trees in different altitudes

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Quercus branti is the most important species in the Zagros forests of Iran. The restoration this species is hard, so identification this species help us to restore plans with a higher success percentage. Leaves of fifty trees of *Q. branti* were sampled for morphological studies, from different altitudes (700-2700m) in a province of Iran. Every 500m height was considered as an altitude level, so 5 altitude levels were considered. Then a comparison of means was made between these levels by ANOVA analysis.

Results showed that there were a gradual decreasing of total leaf length, length of lamina, maximal width of lamina, height of maximal width, number of lobe and intercalary veins on right and left, dry weight, leaf area, volume, length of lamina from base to widest part and HMW/MWL from low altitudes to high altitudes. But

there were most lobe width, distance between midvein and sinus base and distance between midvein and lobe apex in 700-1200 and 2200-2700m altitude.

This study shows that it seems 1200-1700m altitude level is best habitation for *Q. branti*.

P2105. Patterns of plant attributes in the Pyrenean Alpine vegetation

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We seek the degree of adaptation of plant communities to environment through comparative analysis of functional attributes. From all the available relevés (about 800), we have characterized each community (47 associations or subassociations) in terms of 11 attributes. A fuzzy multivariate classification based on these attributes gave 7 groups and 5 sub-groups of plant communities, most of them coinciding with high syntaxa, and corresponding to main Alpine habitats. With respect to the whole Alpine flora, the most differentiated groups include species-poor communities, frequently bearing high percentages of particular types (like chamaephytes, succulents, lichens or endozoochory), and are related to a variety of habitats, but in all cases associated to stress (scarcity of soil, unfertile or water-logged substrata). Groups less differentiated from the global spectra include in the main richer communities, with more shared dominances, and correspond to more balanced environments.

P2106. Distribution and environmental correlates of plant functional types in Germany

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Ecological traits could act as surrogates for functional characteristics of plants. There are many theories linking plant functional types to environmental characteristics. Understanding such relationships will add on the knowledge of ecosystem functioning. Rarely, however, were such plant functional types brought into space to analyse distribution patterns of such types in relation to environment.

We analyse the relationship between functional diversity or relative abundance of plant traits such as pollination syndromes and ploidy levels with environmental covariates, recognising their spatial structure. Data sources are the database BioFlor on biological and ecological traits of the flora of Germany, the distribution database of vascular plants in Germany (FLORKART) and selected environmental variables considering geology, land use and climate. Thus we are able to provide an area-wide distribution map of functional types (or functional diversity) and their relevant environments.

P2107. Many singers, one song: association between plant strategies and ecological indicators

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British comparative plant ecology has used experimental approaches to measure innate traits under standardised conditions and to derive plant 'strategies' or functional types ($n > 1010$). In contrast, a Central-European approach has emphasised the unity of plants with their natural habitats by means of indicator values (German: Zeigerwerte) which describe their ecological behaviour in the field ($n > 2700$). Here, we identify levels of compatibility/congruence between these two approaches using large datasets that include some previously unexamined traits. Despite there being a wide gap between the plant- and environment-based starting points, we discover that both lead to similar conclusions regarding evolutionary tradeoffs and ecological processes. In particular, the results add support to a theory of evolutionary specialization wherein plant life has to choose between those traits which confer rapid acquisition of resources and those which confer their long-term conservation.

P2108. The role of plant functional traits in determining the vegetation composition of abandoned farmlands on the Delatite peninsula, Victoria, Australia.

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In the Northern Hemisphere species with short distance dispersal mechanisms are typically absent from abandoned farmland due to the time it takes these species to re-colonise from adjacent forests. In Australia little is known about the dispersal mechanisms and other traits of species affected by clearing for agriculture and subsequent abandonment. This study aimed to identify species' attributes associated with sensitivity to change in land use and the ability to re-colonise abandoned farmlands on the Delatite peninsula. Past agricultural land use was found to have significant effect on the present day vegetation composition when compared to adjacent native forests. The frequency of life forms and dispersal mechanisms in native species varied significantly according to past land use. These results show that species with limited dispersal ability have generally failed to re-colonise abandoned farmlands, consistent with results from the Northern Hemisphere.

P2109. Biological Characters and Application of 47 Species Climbing Plants

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By observing the growth, blossom and fruit habits, phenological period of 47 species climbing plants, we have known the yearly net growth length, the fast growth stage, blossom and fruit characters, defoliation or evergreen habit, the climbing style of every species. All these characters are the important reference to apply rationally them to making the special environment green.

The results of application experiment showed that the species with evergreen and strong drought-hardiness characters, such as *Macfadyena unguis-cati* A., *Pueraria montana* Merr. and *Bauhinia corymbosa* Roxb. etc., are the very powerful materials that can be used to afforest the stone surface of transportation slope protection and barren hill. *Indigofera eneaphylla* L. Linn. is a good material covering the bare loess slope and the soil near the root of the tree. The rational distribution of *Pharbitis india* R.C.Fang, *Pyrostegia venusta* Miers and *Thunbergia laurifolia* Lindl with *Parthenocissus heterophylla* Merr and *Ficus pumila* L. can not only make the motorway interchange green but also beautify it.

P2110. Understorey tree-ferns: Filters or indirect facilitators for the establishment of canopy species in a New Zealand conifer-broadleaved forest.

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In a New Zealand conifer-broad-leaved forest, we tested the competitive and facilitative effect of the tree-fern understorey of *Cyathea smithii* and *Dicksonia squarrosa* on the regeneration of canopy angiosperms and gymnosperms.

We surveyed epiphytic seedlings growing on fallen and standing ferns and correlated seedling height and abundance with light availability. Tree seedlings were found to be significantly more abundant on fallen tree-ferns than on standing tree-ferns. However, ones found on standing tree-ferns were significantly taller. Correlation between epiphytic regeneration and species establishment showed that more than 45% of the adult trees established on standing tree-ferns.

We quantified changes in seedling growth of 4 species of angiosperms and gymnosperms transplanted on the forest floor with and without the tree-fern layer.

Tree-fern layer removal lead to significant increases in RGR of the 4 species. In contrast, RGR of epiphytic seedlings was not affected by tree-fern removal.

Species regeneration is not only determined by competition between the forest layers, but also but also by indirect facilitation.

P2111. Litter deposition in a fragmented area in the Brazilian Atlantic Forest

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Plant litter plays an important role in tropical forest ecosystems and to the maintenance of diversity, including insects, soil micro-organisms and vegetation. We investigated how litter is distributed in four fragments of different size and degrees of isolation by

agricultural activities. We selected fragments isolated ($F_3= 3.2$ ha; $F_4= 62$ ha) and connected ($F_2 = 8$ ha; $F_1= 23$ ha). Litter traps (16) were established at four distances from the edge. ANOVA, Tukey post-hoc test, correlation, PCA, ordination and cluster analysis were carried out to define the relationships among fragments. The average amount of litter supplied was 13.5 t ha^{-1} similar to other studies in Atlantic Forest. Leaves represented 60.4 %, branches (16.2%) and (6.5%). Neither distance from the edge nor isolation degree affected the litter deposition pattern along the period. Fragments' size was the most important variable separating biomass production among fragments. (*Blumen Project supported by CNPq and BMBF-Germany Agreement- <http://www.tt.fh-koeln.de/blumen>*)

P2112. Frugivory and seed dispersal by birds in an altitudinal Atlantic Forest, Teresopolis-Brazil

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Effective seed dispersal is critical to restoration of degraded areas and to successful recruitment in fragmented forests. We investigated seed dispersal by birds in and among four fragments of different sizes and degrees of isolation by agricultural activities. Fragments isolated ($F_3= 3.2$ ha; $F_4= 62$ ha) and connected ($F_2 = 8$ ha; $F_1= 23$ ha) were studied by mist-netting in two sites per fragment. Bird captures were performed; seeds were collected from feces and germination was carried out in controlled chambers at 30°C . Frugivores birds were more abundant (38.3%; $n= 31$), followed by omnivores and insectivores. Seeds of *Miconia* and *Lendra* sp. were the most frequent and were dispersed by *Chiroxiphia caudata* and *Thraupis sayaca* that promoted increasing of seed germination while *Hemitriccus diops* and *Turdus leucomelas* were not effective in breaking seed dormancy. (*Blumen Project supported by CNPq and BMBF-Germany Agreement- <http://www.tt.fh-koeln.de/blumen>*)

P2113. Seed bank as bio-indicator of fragmentation in an altitudinal Atlantic Forest- Teresópolis, RJ- Brazil

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Although seed banks affect successional patterns in communities, few studies have investigated the demographic relationship between fragmentation and seeds in the soil. We examined the spatial and temporal variation of seed bank in four fragments surrounded by agricultural activities. We selected fragments isolated ($F_3= 3.2$ ha; $F_4= 62$ ha) and connected ($F_2 = 8$ ha; $F_1= 23$ ha) of different sizes (small < 10 ha; larger > 15 ha) in an altitudinal area of Atlantic forest in Teresopolis (RJ). Seed traps (16) were established at four distances from the edge. ANOVA and correlation were carried out to study relationships among fragments. Soil samples (10 cm depth) from eight plots (0.25 m²) were collected at four distances from the edge. Seed germination from soil samples was performed in a nursery. The mean density was 102 seed m⁻². Fragment size and seed rain were correlated ($r^2 = - 0.96$); larger fragments showed less seed germination than the small. Seed bank increased from edge to 30 m decreasing after this, except in the largest (F_4). Pioneer seeds were dominant; seed bank could be used as bio-indicator of fragment degradation in studied area. (www.tt.fh-koeln.de/blumen)

P2114. Seed rain as bio-indicator of forest fragmentation in an altitudinal area of Atlantic Forest, Teresópolis, Brazil

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Although forest fragmentation in Brazilian Atlantic Forest is a well-known problem, few studies have investigated the demographic patterns of seed rain as an environmental indicator of recovering or degradation. We studied the relationship between seed rain diversity in four fragments surrounded by agricultural activities. We selected fragments isolated ($F_3= 3.2$ ha; $F_4= 62$ ha) and connected ($F_2 = 8$ ha; $F_1= 23$ ha) of different sizes. Seed traps (16) were established at four distances from the edge. Correlation, PCA, ordination, species indicator and cluster analysis were applied to define the relationships among fragments. *Miconia* sp. and *Cecropia* sp (172.9 seeds/m²), represented 24 and 29% of the total seed input and were concentrated on 35-65 m from the edge. The largest fragments showed more similarity (F_4 and F_1) and were grouped with F_2 , and the small one (F_3) was completely isolated from the others. All fragments showed low diversity ($H' \text{ m}2.0$) of

seed rain, with high density of pioneer species. Bat dispersal dominance indicated their importance to seed rain. Isolation could be more related to seed rain diversity. (*Blumen Project* - www.tf.th-koeln.de/blumen)

P2115. Fruiting phenology in an understory community in Manaus, Central Amazonia.

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Fruiting patterns are directly related to tropical biotopes dynamics. The understory, often neglected, is a species-rich layer and a source of food for the fauna. In this study, the fruiting patterns of an understory community were characterized, within 5m from the ground, in a terra firme forest in Manaus, Brazil. The field observations were made along four transects covering one hectare, from August 2003 to December 2004. A total of 40 species from 18 families were observed, producing 85% of fleshy fruits (drupes and berries) and 15% of dry fruits (caripose, nut and capsule). The most common fruit colours were green, black, brown and purple. Rubiaceae(10sp.) and Piperaceae(6sp.) were the richest families. All species produced fruits adapted to biotic dispersal, with 37(92,5%) species possibly endozoochorous. In relation to fruiting time, 46,2% of the species produced fruits for more than five months, 30,7% up to five and 23,1% up to four months. The understory community showed a continuous fruiting period, with at least 8 species fruiting per month. Results reaffirm the complex and constant biotic interaction that exist in the understory.

P2116. Re-colonization of experimental gaps by terricolous bryophytes in pine forests

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We studied re-colonization patterns of experimental gaps by four dominant bryophyte species over three years in north-eastern German pine forests. Vegetation and litter layer were removed on 1 m² plots within ± pure bryophyte colonies. All plots were re-colonized by the species which was dominant before gap creation. Three vegetative mechanisms interacted: (1) advance of surrounding shoots from the edge, (2) dispersal of detached shoots resulting in new colonies by continuing growth, and (3) regeneration from seemingly dead stem fragments in the humus layer. *Scleropodium purum* displayed the most rapid growth. Due to their larger vegetative diaspore bank, colonization of *Hypnum jutlandicum* and *Dicranum scoparium* was at least as fast than of *Pleurozium schreberi*, despite lower rates of advance from the edge. The different clonal colonization strategies account for the high competitive capacity and regeneration potential of the four bryophyte species despite the lack of generative reproduction. Experimental disturbance also resulted in a temporary increase of bryophyte diversity, because short-lived colonists appeared in the gaps.

P2117. *Pinus strobus* invasion spread in the Bohemian Switzerland Sandstones

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Pinus strobus is an invasive tree in the sandstone areas in the Central Europe. In the Bohemian Switzerland, we focused on distribution and demography parameters of *P. strobus* self-sowings in different habitats with respect to the distance of the diaspore sources. The data were confronted with a theoretical dispersal modelled on the basis of a seed terminal velocity and a known wind speed. We found that (i) *Pinus strobus* density sharply decreased up to 50 m from the seed trees and approached nearly zero at the distance of 100 m. It corresponds with the seed dispersal under the wind with the speed below 6 m/s, prevailing in the area, and with the short-range dispersal that effects most diaspores but is less relevant for driving the spatial spread. (ii) Only small fraction of the self-sowings was found further than 100 m suggesting there is only rare long-distance dispersal. Nevertheless, it may be the driving force of the whole invasion process. (iii) The significant differences in regeneration in the different stands and habitats along the sandstone microclimate gradients were found.

P2118. Application of cellular automata modeling for plants invasion model

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The models of occupation of new territories by invasive plant populations are formulated on the basis of the concept of discrete description of plant ontogenesis using cellular automata technology. The comparative rates of expansion by the autochorous and zoochorous type of dissemination have been analyzed. Species of herbaceous plants with different strategies: competitor (*Aegopodium podagraria* L.), reactive (*Stellaria holostea* L.) and stress-tolerant (*Asarum europaeum* L.) species were chosen. The model is based on the field experimental data collected in the "Bryanskii Les" State reserve in the centre of European Russia. The analysis of invasion rates on the bare territory by modeling population dynamics (number, age state spectrum) in dependence of a) combination of dispersal types; and b) heterogeneity of territory (different correspondence between empty and busy sites) was made.

P2119. Constructing biogeography: using simulation studies to investigate historical biogeography

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Simulation studies may be useful for investigating historical biogeography, however such methods have not been explored widely. Here we describe a simulation method for examining the effects of various parameters on the realization of specific biogeographic patterns within a phylogenetic framework. Specifically, populations are evolved within a spatially explicit scenario, with the history of the lineage recorded as a phylogeny that can be compared to expected patterns. Simulations are modeled at the level of the individual, which also allows the effects of species abundance on speciation and competition to be considered. The approach is very flexible allowing many different scenarios to be addressed. For example, the spatial relationships between areas may change during the simulation so that the influence of specific events (e.g., continental movements or vicariance) on the resulting phylogeny to be investigated. We describe several hypothetical and a southern hemisphere example. These results suggest that simulation studies can be an appropriate tool for examining biogeographic patterns.

P2120. Genus *Taraxacum* Wigg. in Poland. An attempt of geographic and ecological analysis

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Up till know, 368 species of genus *Taraxacum* have been observed in Poland. Basing on the maps of dandelion distribution in the 10 km² squares, and present ecological data, an attempt was made of classification of some species to geographic groups, determination of their site preferences, and specification of endangered species.

The classification of Polish dandelions into geographic groups: endemic species - *Taraxacum polonicum*, *T. pieninicum*, *T. glowackii* (?), and *T. podlachiacum* (?); Central European species - *T. subalpinum*, *T. paucilobum*, *T. subdolum*, *T. mendax*, *T. skalinskianum*, *T. turfosum*, *T. trilobifolium*, *T. subpolonicum*, *T. ancoriferum*; Atlantic species - *T. hollandicum*, *T. udum*, *T. lancidens*; Subatlantic species - *T. brachyglossum*, *T. disseminatum*, *T. gracilinum*; continental species - *T. bessarabicum*; submediterranean species - *T. erythrospermum*. Endangered species: Ex - *Taraxacum madidum*; E - *T. balticum*, *T. boekmanii*, *T. bracteatum*, *T. lancidens*, *T. nordstedtii*, *T. piennicum*; R - *T. ethiops*, *T. blomgrenii*, *T. corynodes*, *T. fasciatum*, *T. hamatum*, *T. leptoglotte*, *T. maculatum*, most of them belonging to the section *Palustria*.

P2121. *Eragrostis albensis* H. Scholz - a species spreading on anthropogenic sites in Poland

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Eragrostis albensis H. Scholz was described as a species new to science in 1996. In the area of Poland, it was found mainly in the valleys of the Vistula and San Rivers and at first treated as *E. pilosa*. A lot of new stations of *E. albensis* were found in the south-

eastern part of Poland, in 2003-2004. The species occurs there in anthropogenic habitats e.g. railway-tracks, platforms, reloading areas near the railway stations, roadsides and gaps between flagstones. In many cases, *E. albensis* forms homogeneous patches with other plants which could be documented in phytosociological relevés. More than half of the relevés included *E. minor*. The results of the research have proved that we witness expansion of *E. albensis* in the anthropogenic habitats in Poland. This is the effect of biological-ecological properties of the species and anthropopressure. *E. albensis* will probably spread in Poland just like *E. minor*. Both species have the same ways of dispersal (boleochory and anemochory). The distribution of new stations of *E. albensis* in Poland, diagnostic characters, phytosociological documentation and hypotheses concerning its spread are presented.

P2122. Postglacial population expansion of *Dacrydium pectinatum* (Podocarpaceae) in Hainan, southern China, based on cpDNA *trnL-F* noncoding sequence data

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CpDNA *trnL-F* noncoding sequences of individuals of a tropical coniferous species, *Dacrydium pectinatum*, collected from twelve natural populations located in Hainan, southern China, were determined. Thirty haplotypes were identified based on statistical parsimony algorithm. Populations are lack of genetic differentiation. Such a deduction was supported by observed F_{ST} values (= 0.00), high values for Nm (from 1.92 to 2.50), AMOVA (24.17% of molecular variance attributed to difference among populations, $P > 0.05$) as well as the branching structure in NJ tree constructed from haplotypes. A 'star-like' pattern was exhibited in the network of *trnL-F* haplotypes established by statistical parsimony algorithm, and majority of the haplotypes coalesced near the tips in NJ tree. Gene genealogies of cpDNA haplotypes proposed a recent population expansion, which was further lent support by the results from Tajima's D test and mismatch distribution analysis. Our data in junction with geological and palynological evidences showed that in the Holocene, due to global warming, refuge populations of *D. pectinatum* in Hainan might experience a range expansion.

P2123. Distribution, migration and habitat preferences of some newer kenophytes in the territory of Poland

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The natural barriers of kenophytes expansion are overcome by globalization and human impact, therefore their number in the flora of Poland is constantly increasing. Presently, there are about 270 established kenophytes in Poland (approximately 10% of the whole flora of vascular plants). The occurrence of some of the newest arrivals, which were found in the area of Poland during the last 20-30 years, is especially interesting. The specific conditions of their habitats create possibilities for the migration of their diaspores. They establish in new places and in consequence spread, sometimes becoming expansive. These problems in Poland relate to e.g. *Chaerophyllum aureum*, *Eragrostis albensis*, *Typha laxmannii* (entering semi-natural or natural plant communities and achieving the status of hemi- or holoagrophytes), *Brachyactis ciliata* and *Geranium sibiricum* (which are considered as epecophytes). Detailed studies on their distribution, migration, dynamic of population, level of establishing and share in plant communities are required. The results of these studies could be in future of a particular value for the nature conservation programmes.

P2124. Above- and belowground interaction of wheat (*Triticum aestivum* L.) and wild oat (*Avena fatua* L.)

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A pot experiment was designed to investigate above- and below-ground interactions of wheat with wild oat. This experiment was conducted in a randomized complete design with four treatments and four replications. The treatments include above-ground, below-ground and full interaction of wheat - wild oat and no interaction of wheat and wild oat. In both plants, full and belowground interaction decreased plant height, number of leaves, flag leaf area, chlorophyll content of flag leaf, biomass and grain

yield compared to aboveground interaction and control, significantly. In wheat no significant difference observed between belowground and full interaction except wheat biomass and grain yield. All traits of wild oat reduced by aboveground interaction compared to control. In full interaction, flag leaf chlorophyll content of wheat and wild oat were reduced about 10.7% and 6.9% compared to control, respectively. Number of leaves, biomass and grain yield of wheat and wild oat showed greatest susceptibility to interaction treatments. Wheat and wild oat plant height increased by aboveground and full interaction related to no interaction, respectively.

P2125. Cacti species conservation in central Mexico. A case study in the Biosphere Reserve "Barranca of Metztlán"

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The illegal extraction of plants, goat overgrazing, and deforestation have led to a great environmental disturbance in central Mexico. The "Barranca de Metztlán" (Biosphere Reserve since 2000) possesses 62 cacti species (18.6% of all the Mexican cacti). Most of the cacti species are used for local people as food, fodder or living fences. This study demonstrated that *Prosopis laevigata* and *Senna wislizeni* (Leguminosae) form resource islands, not only rich in soil nutrients but in arbuscular mycorrhizal fungi, and perform as nurse plants, while the cacti (*globose) **Echinocereus cinerascens*, **Mammillaria longimamma* (endanger species), **M. magnimamma*, and **M. obconella* are nursing plants during their whole life cycle, and *Myrtillocactus geometrizans* is associated to the nurse during its first stages of life; while *Cylindropuntia imbricata* and *Opuntia streptocantha* establish better in open areas. *Prosopis laevigata* and *S. wislizeni* should be considered for ecological restoration practices within the Reserve. Conservation strategies should consider both: nurse plants and cacti, as well as the systems of management exercised by local people.

P2126. Floristic similarities between lowland and montane areas of atlantic coastal forest in northeastern brazil

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Using a binary matrix, and TWINSPLAN analysis intends to analyse floristic similarity. Were performed on 742 tree/shrub species listed in 35 different plant surveys. These tree/shrub were divided by these analysis into two large floristic groups ombrophilous/semideciduous. The semideciduous group was formed, by forest areas located at altitudes above 700m, and could itself be divided into two subgroups. The first subgroup was located more inland, while the second subgroup was located nearer the coast. The ombrophilous group was quite heterogeneous, but could also be divided into two floristic subgroups: i) lowland forests in the states of Pernambuco, Paraíba, and in two areas of Bahia, as well as some montane forests; ii) lowlands forests in the states of Alagoas and Bahia. Nonetheless, it can be further subdivided into two smaller classes, one in Alagoas and the other in Bahia. Was demonstrate that the concept of the Atlantic Coastal Forest could also include montane ombrophilous forests.

P2127. Analysis of life forms in flora of Divcibare

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Divcibare is part of Mt Maljen which is situated in central part of western Serbia. Geological layer is serpentine. The vegetation consists of forests and mountainous meadows and pastures. During floristic investigation we have performed ecological analyses of this area through determination life forms for each species. We could conclude that hemicriptophyta (H) are the most numerous with 260 taxa (63.35 %) in flora of Divcibara. The geophyta are presented with 46 taxa (11.16 %), terophyta with 45 (10.92 %), phanerophyta with 26 (6.31 %), chamaephyta with 20 (4.85 %), scadentophyta with 7 (1.70), parasitophyta and semiparasitophyta with 5 (1.21 %) and hydrophyta with 2 taxa (0.48 %).

Hemicriptophyta are the most numerous in flora of Balkan peninsula, generally. Number of geophyta, terophyta and chamaephytat in flora of investigated area confirms that investigated vegetation is not high mountainous vegetation.

P2128. Age dependence flowering of *Thalassia testudinum* in Champotón, Campeche and Celestún Yucatán, MéxicoM. E. Gallegos¹, M. Merino², F. López¹;¹Universidad Autónoma Metropolitana Iztapalapa, México, Distrito federal, México, ²Universidad Nacional Autónoma de México, México, Distrito federal, México.

We determine the age dependence of flowering events and flowering intensity of *Thalassia testudinum* along coast of Champotón, Campeche (19°02' N; 96°04' W) and Celestún (20°45' N; 90° 25' W) Yucatán, in the Gulf of Mexico, using the reconstruction techniques (Duarte et al 1994). In Champotón only 17 % of the shoots examined had flowered during their lives, and the mean flowering frequency for the population was estimated to be 6.7×10^{-3} flowers per plastochrone interval (PI⁻¹), which corresponds on average to a flowering event every 7 years. None of the shoots examined flowered before reaching an age of 21.5 PI (about 1 year). In Celestún only 12.59% of the shoots examined had flowered during their lives, and the mean flowering frequency for the populations was estimated to be 4.7×10^{-3} flowers per plastochrone interval (PI⁻¹), which corresponds on average to a flowering event every 9 years. None of the shoots examined flowered before reaching an age of 22.7 PI (about 1 year).

P2129. Effect of plant growth regulators and N, P, K fertilization on leaf pigments and other cell metabolites in shatavari (*Asparagus racemosus*)

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Asparagus racemosus, is a climbing plant which grows in low jungles areas throughout India. *A. racemosus* (Willd.) is an important plant of female fertility of the monogeneric family Asparagaceae. This tonic herb is life supporting or sattvic in its nature. Influence of different growth regulators and N, P, K were studied in the present study. One month old seedlings grown in pots were exposed to 12 foliar sprays of different growth regulators viz. IAA, IAB, NAA, GA3 and N, P, K in the form of urea, super phosphate and muriate of potash respectively of different concentration at an interval of 15 days. The control plants were sprayed with distilled water. All the treatments promote significant increase in the total biomass plants. Photosynthetic pigment was found to be maximum in the IBA and nitrogen treated plants among all the treatments. Phosphorus application at 160 mg/kg and IBA application at 50 ppm concentration significantly increased the contents of starch, reducing and total soluble sugars. The total soluble protein contents were also consistently more in all the treatments as compare to that of control plants.

P2130. Soil microbial community of a central Siberian *Pinus sylvestris* forest

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Structure of microbial community in sandy soils of a central Siberian *Pinus sylvestris* forest was investigated. There were two closely located areas: one was covered with trees (referred to hereafter as "forest") and second was an open area of around 225 m² (referred to hereafter as "glade"), for which the ground cover consisted of only by lichen species. The microbial-community structure was determined on the basis of a PLFA pattern within 100 cm soil profile in the middle of vegetation period. Thirty-eight microbial fatty acids were identified by GC-MS and used for a principal component analysis (PCA) to detect the composition differences.

PLFA-profiles of soil microbial community of both areas ("forest" and "glade") were similar at 95%. There were some fatty acids (FA) peculiar to Gram-negative-bacteria in microbial community composition of "forest" soil only. In upper layer of 'glade' soil it was found FA peculiar to protozoa. It was detected a remarkable shift (20 cm deep down) in PLFA-profiles of the "forest" against the "glade" distribution of microbial community.

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P2131. Redlist of the Hungarian vineyard weedsR. Pál¹, G. Pinke²;¹Research Group for Biological Adaptation, Hungarian Academy of Sciences – University of Pécs, Pécs, Hungary, ²University of West Hungary, Department of Botany, Mosonmagyaróvár, Hungary.

Many reports have been published in Europe (Germany, Switzerland) about decreasing biodiversity of vineyards recently. The weed flora of the Hungarian vineyards is highly influenced by intensive growing technologies (high mulching intensity, frequent soil movement, use of herbicides) as well. Abundance of sensitive therophytes (*Androsace maxima*, *Calepina irregularis*, *Medicago arabica*, *Thlaspy alliaceum*) and bulbous geophytes (*Gagea arvensis*, *Muscari racemosum*, *Ornithogalum boucheanum*, *O. umbellatum*) are decreasing. Frequency of invasive newcomers (*Conyza canadensis*, *Erigeron annuus*) and stoloniferous plants (*Convolvulus arvensis*, *Cynodon dactylon*, *Elymus repens*) showed however a great upsurge. Rare weeds can only be found on extensive managed fields, some of them are legally protected and some are listed in the Red Data Book of Hungary. Our aim is to assemble a list of the endangered weed species of the Hungarian vineyards. There are already preservation programs going on to protect rare weeds in some European countries. We would like to contribute with our list to possible introduction of similar programmes in the Hungarian winegrowing areas.

P2132. Photosynthetic efficiency of seeded *Festuca rubra* L. and spontaneously recolonized *Calamagrostis epigeios* (L.) Roth. at fly ash deposit of "Nikola Tesla - A" thermal power plant in Obrenovac (Serbia)M. M. Mitrovic¹, P. Z. Pavlovic², D. V. Lakusic³, L. A. Djurdjevic¹, S. Bojovic¹;¹Department of Ecology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia and Montenegro, ²Department of Ecology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia and Montenegro, ³Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia and Montenegro.

The paper analyzed photosynthetic efficiency of PSII (Fv/Fm) of *Festuca rubra* L. and *Calamagrostis epigeios* (L.) Roth. at fly ash deposit (FAD), which covers an area of 400 ha. *F. rubra* was the component of grass mixture used for revegetation of deposit. *C. epigeios* was populated by natural recolonization. The objective of research was to evaluate the vitality of two grasses at deposit in relation to natural site-control (NS), and coordination of their ecological potentials with the potential of ash deposit.

F. rubra was abundant at FAD, with mean Fv/Fm of 0.690±0.078 (up to 0.770). Higher values of 0.757±0.044 (up to 0.829) were measured at NS (p<0.01). *C. epigeios* formed a dense canopy cover at FAD, with mean Fv/Fm of 0.581±0.115 (up to 0.779) and of 0.668 ±0.071 (up to 0.784) at NS (p<0.001). Results showed higher Fv/Fm of *F. rubra* than of *C. epigeios* (p<0.01), and maximum values within the optimum range (0.750-0.850) in both species. According to its vitality *C. epigeios* should be included to grass mixture. Such vegetation established on ash deposit would provide its efficient protection from wind erosion, with minimum investment and without special land management.

P2133. Nitrogen fixing activity of diazotroph microorganisms associated to *Rhizophora mangle* and *Avicennia germinans* in the Colombian CaribbeanJ. Vanegas Guerrero¹, J. H. Polania², J. Sánchez Nieves¹, A. Lozano de Yunda¹, H. Valencia Zapata¹, T. Galindo¹, L. M. Melgarejo¹, N. Moreno¹;¹Universidad Nacional de Colombia, Sede Bogota, Bogota, Colombia, ²Universidad Nacional de Colombia, Sede San Andrés, San Andrés isla, Colombia.

Microorganisms promoting plant growth represent an option to restore ecosystems, and to develop commercial crops in high salinity and/or low nutrient levels soils. This work assessed the nitrogen fixing activity (NFB) of bacteria isolated from mangrove seedlings of two sites at the Colombian Caribbean. For dilutions methods as well as viable cell counting isolations were done sowing rootlet segments directly on semi-solid diazotrophic bacteria media (Nfb). Nitrogenase activity was evaluated by acetylene reduction assay in 16 morphotypes. *Azotobacter vinelandii* (G10) showed best response (56.6 nmole ethylene/culture/hour). Mixtures of this and phosphate solubilizing microorganisms showed no differences in regard to individual nitrogenase activity. *Azotobacter vinelandii* was inoculated in hydroponic Río Grande tomato seedlings under salinity (EC 2.27 dSm⁻¹) during their vegetative phase. After 40 days plant growth was promoted, and best results were obtained in nitrate

concentrations around 16 meq/l. Inoculated seedlings showed differences in their root length with respect to the control, despite of the salinity level.

P2134. Globular cacti microhabitat in a crassicauleous scrub in central Mexico

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Cacti survivorship and establishment are determined by climate and edaphic characteristics in the Mexican crassicauleous scrub. Recently, it was demonstrated that globular cacti such as *Echinocereus cinerascens*, *Mammillaria longimamma* (endanger species), *M. magnimamma* and *M. obconella* may establish below the canopy of legume trees and shrubs, which create resource islands. Although globular cacti populations are more numerous in the resource islands located in the northern slope of the hills than in the southern slope, this study point out that in open areas of both slopes, these globular cacti species are capable of transforming their own microhabitat. Rhizospheric soil has similar nitrogen (30 mgKg^{-1} vs 28 mgHg^{-1}) and phosphorus (23 mgKg^{-1} vs 29 mgHg^{-1}) content as the ones found in the resource islands. In addition, globular cacti are mycorrhizal in both habitats. In open areas, cacti root length is shorter than in the resource islands but arbuscular mycorrhizal fungal colonization is more abundant. The overall of results suggests that globular cacti species transform their microhabitat as long as they establish favoring their survivorship in long term.

P2135. High temperature and accelerated aging effects on spores of different ferns.

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Seeds have a big trade interest. A lot of studies have been published about seeds which determine their resistance and viability. However, in pteridology circles, scant quantitative works have been carried out to determine resistance and viability of the spores, and develop with the results predictive models.

In the current work, carried out nowadays in the Valencia Botanical Garden, we'll show our first results and hypothesis about spore's high temperature resistance of different fern species. We'll show the viability loss patterns and the mathematics equations calculated. The observed conclusion a priori, shows that doesn't exist higher resistance to thermic shocks on xerophyte's spores than hidrophilous species spores.

Also we have established viability loss curves for this species, applying accelerated aging techniques (Vertucci & Roos, 1993). T50 values (time where samples fallen off to a half of its viability) shows significative differences between invasive or cosmopolite species and ferns of restricted ecological habitats.

P2136. Some Hyperaccumulator Species in the Serpentinite flora of Serbia

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Serpentine mass on the Balkans is among the largest in Europe. In Serbia it takes 75% of entire territory because it occurs mostly in mountains massif (Vasic et Diklic 2001.).

The first experiments of nickel contents in soil and in plant material in Serbia, were with endemic serpentinofit *Alyssum margrafii*. The results of total nickel content in eutric cambisol profile over serpentinized peridotit on Goc and Kopaonik mountain (central Serbia) ranged 5.42 and 1.42 mg.kg^{-1} (Obratov et al. 1999.). After that many other genus were investigated such as *Thymus*, *Hypericum*, *Cytisus* etc. in the various parts of Serbia. (Blagojevic et al. 2002, Vrbnicanin et al. 2004). The concentrations of nickel in soil and plant material over serpentinite parent rock were between 0.50 - 5.42 mg.kg^{-1} .

Some other investigations of nickel content in soil and plant material will be presented and compared with literature data.

P2137. *Trichomanes speciosum* Willd. (Pteridophyta, Hymenophyllaceae) in North-Western France: from knowledge to conservation

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With the aim to further establish a conservation plan for the endangered *Trichomanes speciosum* Willd. in North-Western France, the Conservatoire Botanique National of Brest has carried out a study to improve the knowledge about its life cycle and ecology. Indeed, "independent gametophytes" of the fern are able to maintain vegetatively whereas, classically for Pteridophytes, they reproduce sexually to produce the perennial sporophytes generation. Moreover, in the region, sporophytes are only recorded in old wells unlike gametophytes, present in natural habitat. Both reproductive biology and ecology of the fern were examined to search for possible environmental causes involved in the life cycle disruption observed in natural habitat. In the same time, so as to eliminate the deleterious closing of many wells, specifically designed grids were installed on wells sheltering the fronds of *Trichomanes speciosum* Willd.

P2138. The bryophyte flora of Geneva past and present: an analysis of bryophyte distribution in Geneva using GIS.

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An inventory of the bryophytes of Geneva began with the compilation of bibliographic checklists and data-basing of herbarium specimens in G. An active period between 1888 and 1930 saw 5000 bryophyte specimens collected from Geneva and 251 species reported in literature. Recent collecting efforts around the canton provide data on the current presence and distribution of species, leading to the discovery of 20 new species. The completed project database, with 6500 historical literature or herbarium records, and an estimated 10'000 recent collections, will enable us to compare changes in presence/absence of species within the canton, and in species composition for certain well-collected areas over the past 70 years. Geneva has recently undergone a large amount of change in its climate, agricultural practices and in terms of development. Our data also allows us to analyse the bryological flora of Geneva in detail using the GIS and the vegetation cover, topography, land-use, and geology layers that are available for the canton. We present our methodology and preliminary analysis of the bryophytes of the canton and discuss the application of our findings in the future.

P2139. Surface excavations and its role in floristic diversity conservation

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During last century human activities result in destruction of many natural and semi-natural habitats. Considerable amount of plant species has disappeared due to this process. However, some industrial exploitation created important alternative habitats for many threatened plants (sozophytes). This has considerable significance in disturbed urban areas where natural biodiversity diminished due to anthropogenic pressure. In such areas artificial excavations serve as a suit refuges for many sozophytes.

The distribution and occurrence conditions of red-listed vascular plant species were studied on 77 quarries, gravel-, sand- and clay-pits located in Silesia (SW Poland). In researched excavations over 140 sozophyte species were found, mainly from the IUCN EN, VU, NT threat categories. Analyse of the habitat use and type was done to indicate the key environmental factors determining the spatial distribution of the chosen species.

Opencast industrial excavations have an increasingly important role in the conservation of sozophytes. By controlling disturbances and properly managing reclamation undertakings, conservationist can enhance this role.

P2140. Phytosociological characteristics of the sandy desert formations of Caspian shore of Azerbaijan

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This The flora of sandy Caspian shores of Azerbaijan was studied and 114 flowered plant species were identified in this study performed between 1995-1996. The dominant formation was detected to be sandy-desert type vegetation and it was classified and mapped. The phytosociological characteristics and the productivity of the common formations in the area was analyzed. We have shown that the formations of *Phragmisetum*, *Juncusetum*, *Astragaletum*, *Calamogrostisetum*, *Thyphetum*, *Carexetum*, *Salsoletum*, *Suaedetum* were more prevalent while

the formations of *Juncuseto-Glysirrhizetum*, *Ephedretum*, *Kalidetum*, *Alhagetum*, *Tamarixetum*, *Salicornieto-Kalidietum*, *Petrosimonetum* were more localized to defined spots. The productivity of the ground surface and underground (40 cm) phytomasses were (dried grass per 25 cm²) 40-6400 gr and 50-4560 gr, respectively. The effect of changes in the water level on the common plant species and associations was analyzed.

P2141. Wildfire in an Australian alpine ecosystem: its effects on N cycling presented by a unique set of data

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Australian alpine ecosystems have a long history of fire and also distinguish themselves from their northern hemispheric counterparts through different geology, glaciation, climate and soil properties. Recent landscape scale wildfire in alpine regions in south-eastern Australia in December 2002 - February 2003 has provided a unique opportunity to study the effect of unplanned fire on nitrogen (N) cycling. Five transects of approx. 70 m length were established and also sampled before the fire and comprise three vegetation types (grassland, heathland, woodland) as part of an inverted treeline. Total and inorganic N in soil and ¹⁵N natural abundance of soil and foliage were analyzed before and after the fire and a comparison of root N uptake was conducted between burnt and unburnt areas. Wildfire affected the levels of ammonia but not nitrate in the soil and also changed the isotopic composition of foliage and the upper layers of soil. Plant species reacted differently to an altered nutrient situation in the burnt areas which was reflected by rates of N uptake.

P2142. Flora and vegetation of Fatik mountain, Sanliurfa, Turkey

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This study was performed between 2002-2004 to determine the flora of Fatik mountain, Sanliurfa, Turkey. We determined 153 taxa belonging to 25 families and 107 genera. The dominant families are: *Fabaceae* (22), *Asteraceae* (24), *Poaceae* (31), *Apiaceae* (13), *Brassicaceae* (10), *Lamiaceae* (10) and *Caryophyllaceae* (7). Concerning the number of species the major genera in this region are as follows: *Bromus* (7), *Trifolium* (6), *Medicago* (5), *Hordeum* (4), etc. The flora of the region was analyzed according to its geographic elements, living forms and endemism features. The dominating step vegetation was classified and its map was drawn. The vegetation of the field has been exposed to antropogen effects. Seasonal and phytosociological features of the vegetation of the region has been determined. The polydominant formations in the vegetation of the region are *Astragaletum*, *Astragalo-Festucetum*, *Astragaleto-Hordetum*, *Onobrychetum*, *Phlomiseto-Aegilopsetum*, *Saturejeto-Teucrietum*, *Festucetum*, *Hordetum*, *Arrhenathetum*, *Stipeto-Astragaletum* etc. Phytosociological structures of these formations show seasonal aspect and mosaicity.

P2143. Facilitative effects of alpine cushion plants in the Andes of central Chile: an ecophysiological approach

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Alpine cushion plants can modify the microclimate, facilitating the establishment of other alpine plant species. However there are no studies that evaluated if the microclimatic modifications generated by cushions can improve the physiological performance of those species growing within cushions. We characterized soil humidity and temperature, and nutrient content of soil within and outside cushions of *Laretia acaulis* in the Andes of the mediterranean-type climate zone of central Chile. Additionally, we measured the gas exchange and fluorescence parameters of individuals of *Taraxacum officinale* growing within and outside cushions. Cushions maintained milder temperatures, higher availability of water, and higher nutrient content than the bare ground. Individuals of *T. officinale* growing within cushions showed a better physiological performance than those growing outside only at the end of the growing season when the aridity of the environment is high. This suggests that the provision of moisture is a key factor in the facilitation of *L. acaulis* cushions in the Andes of central Chile. Acknowledgments: FONDECYT 1030821 and P02-051-F ICM.

P2144. Micromycetes at arctic soil (Russian territory).

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The investigation of Arctic has been undertaken in preparation for detection of life in extreme environments. Micromycetes were studied at 4 northern zones of Taimyr Peninsula, Kola peninsula, Polar Ural and at some islands which were the polar deserts (high arctic tundra), the arctic, typical and south tundra. It was shown that the number of soil micromycetes ranges from 0.004 to 31.29 thousand CFU (Colony Forming Units) per gram of dry soil. The general reduced to fungal numbers have often been noted in soil along the steep gradient towards from south to the north. Severe conditions of Arctic resulted in the poverty of species, large proportion of sterile mycelium, dominance of *Chrysosporium pannorum* and species of genus *Penicillium*. Shannon index (H) of species diversity oscillates from 0.91 to 4.38. It consequently increases from north to south. The analysis between all biocenoses in the frequency of 178 species occurrence demonstrated that the resemblance between the polar desert and arctic tundra was more higher than with typical and south tundra.

P2145. Dry matter accumulation and nitrogen content of two spring geophytes in a deciduous forest

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Scilla bifolia L. and *Arum maculatum* L. are typical spring geophytes in temperate deciduous forests. An early spring ephemeroïd *S. bifolia* reaches the reproductive phase at the start of its vegetation development. A late spring ephemeroïd *A. maculatum* has delayed and relative shorter reproductive period then *S. bifolia*, and it prolong vegetation growth to the beginning of summer. During the spring period, both species accumulate dry matter in their underground organs. Allocation of dry matter responds to changes in aboveground and belowground mass nitrogen content. N-belowground content decreases at the start of vegetation cycle, when both species mobilize last seasons' accumulated matter. N-shoot content has the same tendency with slighter decreasing trend. A difference in flowering time, reproductive efforts and endurance of period of assimilation and accumulation in belowground plant structures, make differences in nitrogen dynamics. *S. bifolia* has greater N belowground content than *A. maculatum* at the start as well as at the end of vegetation growth, because of differences in reproductive investment between these species.

P2146. Boron content in coffee plantations (*Coffea arabica*) in two locations of Venezuela

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Samples of coffee plants and their respective soils were taken from two venezuelan coffee producing locations [Santa Cruz (8° 29' N, 71° 20' W, 622 m elevation; Mérida State) and Caripe (10° 10' N, 63° 30' W, 1120 m elevation; Monagas State)] to determine their boron content during harvest. A random block design was used, with three repetitions and nine experimental units per treatment. The plant tissue and soil samples were treated by a hot extraction procedure prior to spectrophotometric determination of boron with azomethine-H. Boron content in fruit and leaf samples were 24.96 ± 2.39, 75.86 ± 2.79 µg g⁻¹ dry weight (Santa Cruz) and 9.56 ± 1.12, 29.08 ± 2.15 µg g⁻¹ dry weight (Caripe). These results show significant differences in the boron content between areas and tissues (*p* < 0,001), while the boron content in soils were 9.08 ± 1.38 µg g⁻¹ dry weight (Santa Cruz) and 4.42 ± 0.95 µg g⁻¹ dry weight (Caripe), showing significant differences in the element content among areas (*p* < 0,001). These results indicated higher reference values for the element content in Santa Cruz than in Caripe.

P2147. Halophytic plants communities near Drogobych (Western Ukraine)H. Trzci ska-Tacik¹, M. Woch²;¹Institute of Botany, Jagiellonian University, Krakow, Poland, ²Institute of Botany, Polish Academy of Science, Krakow, Poland.

Halophytic plant communities (*Puccinellio-Spergularietum salinae*; com. *Salicornia europaea* - *Sueda prostrata*; com. *Aster tripolium* s.l.) were found on 3 localities in Drogobych region (Western Ukraine). There are isolated ("islands") localities, up today near unknown. Halophytes grows here on salted sites, connected with the old salt industry. The floristic composition of these communities were given and taxonomic position of some critical species e.g. *Aster tripolium* ssp. *pannonicus*, *Limonium* cf. *gmellini* or *L. hypanicum*, *Sueda prostrata* were discussed. The knowledge about the halophytic flora and vegetation of these and others localities on Western Ukraine is important for maintain or reject the hypothesis of halophytes migration: from shores of the Black Sea (the Pontic Area) towards north-north-west to Central Europe after the last glaciation or from shores of the Baltic Sea to inland salted habitats. The salt production in the Drogobych region was nearly totally stopped about 1995. From that time area of salted sites diminished; that is the threat for halophytic flora and vegetation. Now the significance of presented data is clear.

P2148. Seed germination of two *Salicornia* species from Saudi Arabia.A. Al-Huqail¹, T. A. Al-Turki²;¹Botany Department, Girls's College of Education, P.O. Box 27104, Riyadh-11417, Saudi Arabia, ²Herbarium, King Abdulaziz City for Science and Technology, P.O. Box-6086, Riyadh-11442, Saudi Arabia.

The germination of seeds of two annual halophytic species of *Salicornia* (*S. saudiensis* Al-Turki & Sawrup *ined.* and *S. qassimensis* Al-Turki & Swarup *ined.* (Chenopodiaceae) was examined in collections from eleven populations distributed in the central and eastern (Arabian Gulf Coast) regions of Saudi Arabia. Their responses to a wide range of fluctuating and constant temperatures and to variation of salinity were tested. Seeds of populations from the interior (e.g. Sabkha Al-Waz) and from coastal populations (Arabian Gulf Coast) showed very high germinability (96%-100%) over the whole range of temperatures. The seeds of these populations of both species can germinate at very high salt concentrations (1200, 1500 mM NaCl at 25/15^o C). Soil samples of these habitats were also analyzed.

Keywords: Saudi Arabia, Germination, Alternating and constant temperatures, Salinity, *Salicornia*, Al-Waz, Arabian Gulf.

P2149. The impact of ground water level and soil salinization on different tugai plant communities in the river of AmydaryaS. Y. Treshkin¹, S. V. Kuz'mina²;¹Institute of Bioecology, Nukus, Uzbekistan, ²Water Problems Institute, Moscow, Russian Federation.

The groundwater level equalled to 0.5-1.5 m is required for plant communities such as *Salix songarica*, *Calamagrostis dubia*, *Phragmites australis*, *Typha angustifolia*, *T. minima*, *T. laxmanii*. For communities of *Salix songarica* and *Calamagrostis dubia* the soil salinization and the groundwater level should be minimum within the first three meters - 0.1-0.3% in soils and 0.8-1.2 g/l in the ground water. The groundwater level in 2-2.5 m. is required for *Populus ariana*, *Populus pruinosa*, *Elaeagnus turcomanica*, *Tamarix ramosissima*, *Halimodendron halodendron* and for grasses such as *Trachmithum scabrum*, *Glyzyrrhiza glabra*. The soil salinization under such plant communities can be rather high only in the first meter layer - 0.8-1.5%. The salinization decreases with the depth reaching 0.1-0.7% in the second and 0.05-0.6% in the third meter layer. The groundwater level in 3-5-4.5 m is bearable for: solonchakous vegetation consisting of *Tamarix hispida*, *Lycium ruthenicum*, *Salsola dendroides*, *Halostachys belangerian*; they are tolerant to soil salinization equalled to 1.6-4.5% in the first two meters of the soil layer and 9-23% in some surface horizons (10-20 cm).

P2150. *Crocus albiflorus*, *Euphorbia verrucosa* and *Hypochoeris maculata*: Mycorrhizal colonization and Biodiversity of symbiotic fungiH. Stockinger¹, K. Haselwandter²;¹Organismic Biology, Salzburg, Austria, ²Department of Microbiology, Innsbruck, Austria.

The plant species listed above normally grow in nutrient-deficient meadows and pastures, and hence are negatively influenced and threatened by intense agricultural management practises.

In order to investigate their mycorrhization, roots were sampled at the Gaisberg (1000 m a.s.l.) and the Wimberg (730 m a.s.l.) in the county of Salzburg, Austria, and stained with trypan blue in lactoglycerol following a standard procedure. Soil samples were taken for extraction of arbuscular mycorrhizal fungal (AMF) spores, for determination of plant available P, total soil P and pH.

Samples taken over the vegetation period reveal that roots are constantly colonized by AMF with frequent formation of vesicles and arbuscules. In addition, the plant roots are also colonized by dark septate endophytes. Quantitative data for both types of mutualistic fungal root colonization will be provided together with a qualitative and quantitative assessment of the biodiversity of AMF spores found in soil.

P2151. Seed germination of *Anabasis setifera* Moq. (Chenopodiaceae) from Saudi Arabia.A. Al-Huqail¹, T. A. Al-Turki²;¹Botany Department, Girls's College of Education, P.O. Box-27104; Riyadh-11417, Saudi Arabia, ²King Abdulaziz City for Science and Technology, P.O. Box -6086, Riyadh-11442, Saudi Arabia.

Anabasis setifera Moq., a halophytic perennial shrub in the family Chenopodiaceae, is widely distributed in coastal and inland salt marshes in Saudi Arabia. Experiments were conducted to determine the effect of temperature and salinity on seed germination and its recovery of germination from saline conditions after being transferred to distilled water. Seeds of *A. setifera* were germinated at five alternating temperatures (15/5, 20/10, 25/15, 30/20 and 35/25^o C) and four constant temperatures (5, 15, 25 and 35) and nine different concentrations of salinities (0, 100, 200, 300, 400, 500, 600, 700 and 800 mM NaCl) at 25/15^o C. The seeds germinated best at the relatively low temperatures and inhibited at the higher temperatures. The seeds can germinate at very high salt concentrations (9.5% 700 mM NaCl at 25/15^o C). Seeds recover after being transferred to distilled water and recovery was higher from higher salinity concentrations (94.5% and 75.5% at 800 and 700 mM respectively at 25/15^o C). This result indicates undoubtedly that inhibition of germination in this species was due to an osmotic effect.

Keywords: *Anabasis setifera*, Germination, Halophyte, Saudi Arabia.

P2152. Plant species richness in unfertilized mountainous grassland communities - a function of the soil chemical conditions?

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There is a hump-shaped relationship between plant species richness and pH. Very acidic alpine soils in the Al and/or Fe buffer range are characterized by a relative excess of H-, K-, Al-, Mn- and/or Fe-ions and by a complementary lack of Ca- and Mg-ions. Only calcifuges are adapted to the prevailing nutrient and acid stress. Therefore, species richness is very low. On the other hand calcareous soils in the carbonate buffer range are characterized by an absolute and relative excess of Ca-ions. Only calcicoles are adapted to this disharmonic nutrient supply and the resulting discrimination of individual nutrients. Therefore, species richness is moderately high. Soils in the silicate buffer range, and soils in the upper part of the cation exchange buffer range are generally characterized by a balanced composition of the soil solution and exchange complex. Therefore, both calcifuges and calcicoles can be present at these sites, allowing a high plant species richness.

P2153. The modern state of vegetation cover of the Amudarya Delta and problems of biodiversity conservation

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Studying of the degradation processes of terrestrial ecosystems connected with the intensification of anthropogenic impact is of international meaning. They are especially acute in arid conditions. Here the ecosystems are characterized by specific unsuitability. They cover the wide range of changes beginning from stress conditions and finishing with rebuilding of the ecosystems' structure that lead to their complete degradation. Vegetation is the most valuable kind of biological resources of the Amudarya delta

and is characterized by high biodiversity (655 species that belong to 65 families and 210 genera) and comparatively low productivity. At present time in connection with the changes of ecological situation in Aral Sea region can observe the intensive development of desertification process and process of vegetation cover degradation. Desertification occupy over 40% of the territory of the Amudarya delta.

P2154. Plants and lichens limit the environmental risk related to abandoned asbestos mines

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Vegetation studies have been performed in the Balangero asbestos mine (NW-Italy), which was the most important asbestos mine of W-Europe, used until 1990, and which still represents a source for the air-dispersion of fibres, strongly dangerous for human health. The synchronical phytosociological analysis of the vegetation series has allowed to evaluate the diachronical succession of plant and lichen communities, which cover the asbestos-rich serpentinites of the excavation and discharge areas, limiting the air dispersion of fibres. Few decades are needed for the formation of continuous plant communities which completely contain the air-exposure of the fibres in the spoil-banks. Lichen colonization, even if limited to pioneer stages of development and showing very low cover values, plays a complementary role on the walls of the mining-basin, particularly where asbestos-veins crop-out. The lichen weathering action strongly modifies the chemical composition of the fibres, enhancing their pedogenic transformations and inactivation. Plants and lichens act as spontaneous bioremediation agents in the restoration ecology of the disturbed site.

P2155. Plant Colonization and Succession on Forest Habitats Disrupted by Strip Mining for Coal in the Central United States

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Coal extraction in the United States since the 1930's has disrupted over 2.4 million hectares of natural vegetation and although stringent reclamation controls were placed into law in the 1970's, well over 300,000 hectares remain untreated or unmanaged in such central states as Ohio, Kentucky, and Tennessee. Disturbed soils of abandoned strip mines are characterized by instability, erosion, extreme acidity, and variable nutrient levels which exposes natural vegetation development to multidimensional selection factors. Successional development studies of selected sites have been conducted since 1960 providing insight into adaptive patterns of native colonizers of the genera *Andropogon*, *Rubus*, *Bidens*, *Danthonia*, followed by *Acer*, *Platanus*, *Liriodendron*, and *Quercus*. Hardwood tree growth in basal area tends to accelerate 30 years after colonization and moderation of the initial low pH of soils. Understanding endemic plant succession on these unique habitats provides options to usual reclamation practices.

P2156. The changes in ground vegetation and pine needles chemistry after the wood ash and nitrogen application

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Wood ash experiment, consisting of 24 plots treated with 1.25-5 t/ha of raw wood ash, 180 kg N/ha, and control, was established in SW part of Lithuania in a 38-year-old Scots pine (*Pinus sylvestris* L.) stand in June 2002. The concentrations of the main plant nutrients (N, P, K, Ca, Mg) and some heavy metals (Cr, Cd, Pb, Ni, Cu, Zn) were determined in the moss *Pleurozium schreberi* and current and one-year-old needles 2 years after the application.

Wood ash influenced the concentrations of P, Ca and Mg in *Pleurozium schreberi*. Significantly higher N concentrations were found in the plots treated with N. There were slight changes of K concentrations in ash plots. Ash, containing low amount of metals had no effects on the concentrations of Cd, Pb, Ni and Cu in the moss. The increase trend was detected only for Zn and Cr.

The current and one-year-old needles were not significantly affected by wood ash. Mg and P concentrations increased, K and

Ca have not changed. Addition of N fertilizers increased N concentration in the needles 9 months after the application, and such increase still remains. Not significant increase detected for Zn, Cd and Pb in the needles.

P2157. Biomass formation, nutrient uptake and release in fern stands *Athyrium distentifolium* on deforested areas affected by pollution: comparison with grass stands

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The aim of this study was to compare the role of fern (*Athyrium distentifolium*) and grass (*Calamagrostis arundinacea*, *C. villosa*) vegetation on deforested areas in the Beskydy Mts. (Czech Republic). Fern stands at a lower aboveground biomass production (*A. distentifolium* 194-350 g m⁻²; *C. arundinacea* and *C. villosa* 635 and 471 g m⁻² - mean values) accumulated in it a large amount of nitrogen (*A. distentifolium* 5.8 g N m⁻²; *C. arundinacea* 4.8 g N m⁻², *C. villosa* 5.3 g N m⁻²). At the slower decomposition of fern litter (19-25 % dry mass of leaflets and 18-19 % stalks per year) than that of grass litter (35-54 % leaves, 17-30 % stalks), the release of Ca (54-55 %) and Mg (86-87 %) was faster and the amount of nitrogen immobilized in one year old fern litter reached up to 46 kg N per ha. However, a comparison of soil features indicates less favourable soil conditions in fern stands (lower pH values, Ca contents and Ca/Al ratios) than in grass stands. These data suggest that fern stand formation on deforested sites has not the same positive ameliorative effect on soil environment as that described for both *Calamagrostis* species.

P2158. Fen meadows: Mycorrhizal colonization and biodiversity of typical plant species

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Fen meadows are habitats with high species diversity, covering an area constantly decreasing mostly due to human impact. As a result the plant cover and the soil micro-flora including the arbuscular mycorrhizal (AM) fungi are disturbed. Re-establishment of former conditions derives benefits from knowledge of the soil micro-flora known to improve plant fitness and soil health. Our project aims at the determination of mycorrhizal status of *Allium carinatum*, *Betonica officinalis*, *Filipendula vulgaris*, *Gladiolus palustris* and *Serratula tinctoria*, which are locally endangered. The plant species, which were collected at different stages of plant development in 2003, are colonized by both, AM fungi and dark septate fungal root endophytes, some strains of which can be considered mycorrhizal. Soil parameters like pH, plant available P, total P content and soil water content were measured and spore counts carried out. The fungal symbionts were identified by partial amplification of rDNA.

P2159. Monitoring vegetation of the northern Azov sea maritime spits in the light of modern tectonic and climate changes

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Reinventorial mapping monitoring of 10 northern Azov sea maritime spits was carried out by fixing the modern condition of spits vegetation on the large-scale (1:10000) maps and by comparison with similar previous maps (Popovych, 1936, Postrygan', 1939). It is established that the increase of meadow-halophytic, water-swamp and internal estuary aquatic vegetation on the background of the reduction of solonchak, sand-shell steppe and littoral vegetation during last 70 years testifies the common trends of spits changes - their flooding and washing out. Such changes are connected with specific natural factors - tectonic lowering of a continental crystal plate moving shelf in a zone of the Azov sea coast arrangement, acceleration of a modern stage of the Black sea transgression and anthropogenic-caused reasons - general sea pollution and intensive raising of the world ocean level, caused by global warming. Such tendencies have become menacing for International Wetlands and International Bird Areas integrity, for settlements and recreational institutions of the spits. Monitoring is aimed at a scientific recommendations to protection and use of spits nature.

P2160. Top-down control of plant ectoparasitic nematodes by soil microbes in natural coastal dunes.**A. M. Piskiewicz;**

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This study is a part of the EU-EcoTrain project that examines and compares different mechanisms of control of plant parasitic nematodes in natural vegetation in coastal dune systems.

The objective of our research was to find which top-down mechanism (microbes, predacious invertebrates and nematodes) are responsible for the control of density of the ectoparasitic nematode *Tylenchorhynchus ventralis* in the root zone of *Ammophila arenaria* (Marram grass) in natural foredune systems. We chose *Tylenchorhynchus ventralis* because this species has 80 times lower densities in natural soil in field than in sterilized soil in optimal greenhouse conditions. We tested the hypothesis that ectoparasitic nematodes in coastal dunes can be controlled by microbial antagonists (fungi, bacteria) or/ and predacious invertebrates and nematodes. The results show that the microbes are the most important.

P2161. Research of soil-plant connections on kurgans in Hungary**E. Herczeg,** K. Penksza, A. Barczy, M. Vona, Á. Malatinszky;

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Authors undertook botanical and soil surveys on four kurgans of the Great Hungarian Plain with a Pürckhauer sampler and prepared coenological tables based on the method of Braun-Blanquet. Alkaline and chernozem mosaics characterised the soil in the surrounding area of the kurgan called Csipo-halom. Botanical survey can be well compared with soil examinations. Besides weed species, several plants belonging to natural grasslands could be found as well. Near the peak, *Agropyro-Kochietum prostratae* association occurs. On the lower zone of the slope the association of loess steppe areas (*Salvio-Festucetum rupicola*) can be found, preserving even *Festuca javorkae*. Excess water has got a significant role in soil formations of kurgan Pincés. Drier soil occurs around the top, meanwhile, culture stratum below recent soil is dampening gradually. Kurgan Hegyes was built both of soils once generated on a loess ridge and of soils from salty wet areas. Top areas are dominated by *Agropyron repens* and *A. pectinatum*. Sides of the mound are covered mainly by *Alopecurus pratensis*, while characteristic species in the neighbourhood of the salty area is *Puccinellia limosa*.

P2162. Alterations in the soil seed bank associated with invasive species**M. Gioria,** B. Osborne;

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Alterations in soil seed bank diversity, composition and abundance could be a major factor in determining the invasive success of certain species. Analysis of the seed banks associated with two invasive herbaceous species, *Heracleum mantegazzianum* and *Gunnera tinctoria*, was investigated adopting the seedling emergence method. The diversity of seeds in the seed bank decreased in areas invaded by both species, although the impact varied with the site examined. *H. mantegazzianum* comprised approximately 60 per cent of the seed banks investigated, with a mean of 9,333 seedlings m⁻². *G. tinctoria* represented less than 20 per cent of the invaded seed banks, with a mean of 748 seedlings m⁻². Of the species present in the seed bank, grasses showed the greatest decrease in composition and abundance. In contrast, *Juncus* spp. and some forbs such as *Urtica dioica* and *Ranunculus* spp. seemed to be better represented in the invaded seed banks. Based on a depth-dependent analysis and germination patterns such as time of germination and germination rates, a transient seed bank was confirmed for *H. mantegazzianum*, whilst results for *G. tinctoria* indicated a persistent seed bank.

P2163. Flora of Lower Silesia (SW Poland) - Dynamics and Threats**Z. Kcki,** Z. Dajdok, E. Szczaniak;

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Lower Silesia is a part of SW Poland, it measures 19950 sq. km. Flora of this region counts about 1890 species of vascular plants, 83 % of them are native while anthropophytes make ca. 17 %. During the last century 332 species have become threatened and

63 died out. Endangered plant species represent 29 classes of plant communities. The most numerous are plants characteristic for semi-natural meadows, swards and thermophilous margins, open rock communities and peat-bogs. The most important reason of threat is loss of habitats due to degradation, fragmentation, exploitation and abundance of traditional farming. Apart from the process of extinction the enlargement of flora is also observed, but only three of recently discovered species are native: *Trichomanes speciosum*, *Coleanthus subtilis* and *Crassula aquatica*. The other are kenophytes which make about 10 % of the flora, some of them became invasive and have negative impact on diversity of species and habitats, mainly river valleys.

P2164. Biodiversity decline as a consequence of overgrazing in semiarid grasslands of Mexico**T. J. Arredondo,** E. Huber-Sannwald;

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In semiarid grassland ecosystems, land conversion to agricultural land and changes in grazing regimes are the two most important factors determining biodiversity loss at both the species and genotype level of plants. Semiarid grasslands in Mexico cover around 100000 Km² but are one of the most deteriorated ecosystems of the country as a result of the interacting effects of overgrazing, long periods of drought and increasing rural development. We examined how decades of overgrazing and drought affect the diversity in these grassland ecosystems both at the species and genotype level, the latter of the most abundant grass species *Bouteloua gracilis* and compared these observations with plant community characteristics from areas which were allowed to recover from grazing pressure for the past 20 years. Our results show that a drastic decline in the abundance of the key species changed productivity and soil water dynamics to a larger degree than would have been expected from loss in plant cover alone. Hence, changes in species diversity and genetic diversity of *Bouteloua gracilis* affect community resilience and ecosystem function.

P2165. Effects of climate warming and species richness level on grassland biomass production**H. J. De Boeck,** C. M. Lemmens, I. Nijs, R. Ceulemans;

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Using assembled grassland mini-ecosystems inside greenhouses, we tested for effects of both climate warming (+3°C) and species richness (communities of 1, 3 or 9 species) on plant biomass production. Warming resulted in lower production, both above and below-ground, probably due to increased water stress. Species richness stimulated above-ground biomass production in 3-species ecosystems, while it did not interact with the induced warming. Richness increased root production only in unheated 9-species communities. Below-ground biomass distribution was affected by warming, with lower root production especially near the surface in heated communities. Species richness did not alter root distribution significantly, suggesting little or no increase of below-ground complementarity. Analysis of above-ground biomass showed a slight positive complementarity effect in multi-species communities, however. Species reacted differently to warming and the number of species in the community, but no net selection effects were observed. In conclusion: warming suppressed biomass production, while the influence of species richness was more variable.

P2166. Phenological responses to snow cover and temperature in snowbed species of the Central Alps**K. Hülber**^{1,2}, M. Gottfried¹, K. Reiter¹, G. Grabherr¹;¹Department of Conservation Biology, Vegetation and Landscape Ecology, Vienna, Austria, ²Vienna Institute for Nature Conservation and Analyses, Vienna, Austria.

Cold temperatures and short growing seasons constrain life cycles of snowbed species, which leads to a highly adapted timing of sexual reproduction. Temperature and winter precipitation, the main factors determining growing season length in snowbeds, are predicted to change under global warming, but the degree of impact on phenology will strongly depend on the environmental factor triggering the development of plants. We examined the influences of snow cover duration and temperature on phenology of selected snowbed species.

Phenological development strongly depended on the time of snowmelt. The earlier a plant became snow free the earlier all phenological phases were attained, but the time to reach a certain stage decreased with delayed snowmelt. Plants becoming snow free late in the year required significantly higher night temperatures to reach a given phase with the differences increasing from buds to fruits but there was no clear pattern for total temperature sums. We conclude that phenological development can mainly be accelerated by warm summer nights and that impacts of climate change will be particularly strong if night temperatures increase.

P2167. Responses of Arctic tundra plants exposed to extreme temperature events

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As the IPCC scenarios project extreme temperature events to increase in frequency, we exposed Arctic tundra plots during 8 days to a temperature rise of circa 9 °C, induced by infrared irradiation. Performance, both at plant level and ecosystem level, was improved during the heat wave. However, afterwards, heated plants were more stressed than unheated plants, probably because they acclimated to warmer conditions and experienced the return to ambient as stressful. Nevertheless, if higher temperatures were combined with desiccation, plants were stressed already during the heat wave. As heat waves are often ensued by desiccation, more frequent extreme temperature events in a future climate can alter/harm ecosystem dynamics. Furthermore, differences between species indicate that temperature extremes may play an important role in changing competitive interactions and thus in affecting community composition. As heated plots were a smaller sink compared to unheated plots during the heat wave, more frequent extreme temperature events may shift this biome towards a source and stimulate climate warming through positive feedback.

P2168. Global change impacts plant-insect interactions: effects on tree chemistry and insect performance

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We evaluated the single or combined effects of N deposition, elevated CO₂ and rising temperature on tree nutrient quality and insect performance under different aspects of plant-insect interactions. Our model systems include conifers as well as deciduous trees (spruce, beech, oak, hornbeam), generalist and specialist insect defoliators (two lepidopterans, one hymenopteran) and a tree-killing bark beetle. Insect herbivores do not receive the same level of nutrition from their host species grown under elevated CO₂. CO₂-mediated increases were observed for C-based secondary compounds and non-structural carbohydrates while levels of N decreased. Insects feeding on enriched CO₂ foliage exhibited reduced growth, but the patterns were host species-specific. The effects of increased N deposition on leaf chemistry and insect performance were generally opposite to those of CO₂ enrichment. Rising temperatures stimulated volatiles released from bark and wood thereby modifying the chemical cues that bark beetle encounter during host selection. Thus, tree-insect interactions altered by climate change may substantially threaten forest ecosystem balances.

P2169. Sensitivity of pine stands in the inneralpine dry valley Vinschgau (Italy) against drought and parasitic infection

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Radial cores of 120 trees were obtained from 8 black and scots pine stands (*Pinus nigra* Arn. and *Pinus sylvestris* L., respectively) exposed to soil dryness and parasitic infection. Ring widths were measured at a resolution of 1/100 mm, dated and tree ring chronologies established. Black pines showed a much higher year-to-year variability in ring widths than scots pines (35 % and 27 %, respectively), which indicates a higher sensitivity to changing environmental conditions. Climate-growth-relationships revealed strong correlations between radial growth and precipitation in spring of the current year (especially March) and previous autumn (especially October). Accordingly, striking growth reductions found in several years were linked to drought stress occurring in these seasons. Results also revealed that black pine stands infected by the pine processionary caterpillar (*Thaumetopoea pityocampa*

Denis & Schiff.) showed temporarily reduced growth rates compared to non-infected stands in severe years of infection only.

P2170. Global change and trophic interactions on a boreal mire

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Effects of enhanced temperature and nitrogen availability on vegetation in a boreal, oligotrophic mire were studied in a factorial design experiment, established in 1994.

Data from the period 1995-1998 show only marginal or no vegetation response. In 2003, after eight years of continuous treatment, marked changes were observed. The closed Sphagnum carpet had declined with 68% in the N treatment and 80% in the N x T treatment. The dominant vascular plants *Eriophorum vaginatum*, *Andromeda polifolia*, and *Vaccinium oxycoccus* showed a striking positive response to the N, T and N x T treatments. Parasitic fungi attacking the two dwarf shrubs showed species-specific responses to the treatments. High disease levels, correlating to changes in plant chemistry, and resulting in extensive host mortality were observed in the nitrogen treatment, while enhanced temperature had a negative effect on parasitic fungi. The study shows that initially positive host plant responses may after a time lag be reversed by disease. It emphasizes the importance of long-term experiments in order to understand responses upon climate change and enhanced nitrogen availability.

P2171. Anatomical Evaluation of Tree Ring Quality for Dendroecological Study of Forest Islands of “Campos Rupestres” at Serra do Cipó, Minas Gerais, Brazil.

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At Serra do Cipó, Minas Gerais, Brazil, “campo rupestre” is the main vegetation type. “Campo rupestre” occurs around bedrock outcrops higher than 700 meters in altitude, in sandy, poor and scarce soils. In places protected from erosion, we found “capões de mata” which are islands of forests surrounded by fields. The dynamics of these forests are poorly known, and there is a need for better understanding of their origins. One way to understand this dynamic is the analysis of growth rings. Rings must be annual and well marked which is uncommon in tropical ecosystems. We searched for the presence of good annual rings in 17 arboreal species of 10 families. Branches of young individuals up to 5 meters high were collected and sectioned for histological analysis. Eight of the 17 studied species exhibited growth rings characterized by flat and thickened fibers. *Cabralea canjerana* (Meliaceae), *Guatteria vellosissima* (Annonaceae) and *Leandra* sp (Melastomataceae) showed the best rings while rings of the other species were weaker. These promising results will lead to further dendrochronological analysis of the antiquity of “capões” including their expansion and contraction.

P2172. Prioritizing conservation of medicinal plants in Sub-Saharan African forests

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The demand for traditional herbal medicine is increasing rapidly in Sub-Saharan countries mainly because of harmful effects of synthetic chemical drugs. Harvesting plants for medicines to treat anything from mad cow disease to malaria could help alleviate the poverty of African communities. The recent upsurge in the use of herbal medicines has led to enormous commercial possibilities but many key issues remain unresolved.

However, the future of African medicinal plants lies on enhancing people's participation in the utilization and conservation of its forests. Worldwide a total of at least 35000 plants species are used for medicinal purposes. The subcontinent has a rich floral diversity, totalling about 24 000 species, with 4 000 species used in traditional medicine.

Protection and preservation of traditional medicine, as well as African Sub-Saharan tropical forests are essential to ensure

access to traditional forms of health care. Programme activities need to be supported to reduce the rate of deforestation of the African tropical forests and conserve the biodiversity contained within them.

P2173. Plant diversity, anthropisation and invasives in tropical rainforests: use of the filmy fern (Hymenophyllaceae) community as a bio-indicator in La Réunion island.

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To study the impact of human activities on indigenous plant diversity in the La Réunion endangered hygrophilous forest, we compared, in natural and exploited plots along an altitudinal transect, taxonomic diversity and ecological preferences of Hymenophyllaceae as representing a major sciaphilous hygrophilous epiphyte community in this type of forest. Regression analyses between taxonomic variables and environmental predictors evidence a significant decrease in filmy fern diversity in anthropised areas. Additional analyses performed specifically on the most abundant taxon do not reveal an anthropisation effect on its fertility despite a decrease in the number of colonies. The decrease in Hymenophyllaceae diversity might be rather due to a decrease in diversity of appropriate supports. In open anthropised plots, invasive heliophilous pests actually delay or avoid the natural rejuvenation of indigenous trees including potential supports for indigenous sciaphilous hygrophilous epiphytes.

P2174. Genetic Variability of *Quercus humboldtii* (Roble) from the “Macizo Colombiano” using RAPDs Markers.

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The genetics impacts of population fragmentation depend upon gene flow among fragments. With restricted gene flow fragmentation leads to greater inbreeding and loss of genetic diversity within fragments. There is genetic differentiation among fragments and greater risks of extinction in the long term, than for a single population of the same total size. This populations involves some species like *Quercus humboldtii* (roble).

In this study RAPDs markers were used to estimate the levels of genetic diversity in 120 individuals from four populations in the “Macizo Colombiano” (Colombia Andes). A total of 123 loci were obtained for all populations using 6 primers, 122 of which were polymorphic. Observed heterozygosity was 0.1172 and Gst 0.1185. Based on the results presented in this study, we recommend that a small number of populations represented by large numbers of individuals will be enough for breeding and gerplasm conservation purposes, and major attention should be paid to population Riñonada for conservation.

P2175. Vegetation survey of threatened alder (*Alnus glutinosa* (L.) Gaertn. ssp. *barbata* (C. A. Mey.) Yaltirik) forests in the Caspian lowlands, northern Iran.

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The lowland forest zone, one of the altitudinal zones of Hyrcanian forests in northern Iran is characterized with small fragmented remnant alderwoods, dominated or subdominated with an Euxino-Hyrcanian element, *Alnus glutinosa* (L.) Gaertn. subsp. *barbata* (C.A. Mey) Yaltirik. As these forests are isolated remnants of a formerly more widespread lowland habitat, there is no exact information available on their vegetation and flora make-up. Alder communities subjected to Analyse Factoriel des Correspondance (AFC) and Classification Ascendant Hierachique (CAH) with ANAPHYTO software and synsystematically treated following the Braun-Blanquet approach. Analysis of 133 relevés from eight different studied sites resulted in six vegetation units which ecologically extend from very wet to rather dryer habitats. *Alnus glutinosa* subsp. *barbata* together with *Thelypteris limbosperma* (All.) Fuchs, *Ulmus minor* Miller, *Carex acutiformis* Ehrh., *Populus caspica* Bornm., *Pterocarya fraxinifolia* (Poir.) Spach, *Pteris dentata* Forsskahl, constitute these six communities. Also the

delimitation of relevant associations and upper syntaxa are discussed.

P2176. The evolution of the *Pinus sylvestris* L. area in the Iberian Peninsula from the last maximum glacial (21000 BP) to 2100 under climate change

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The *Pinus sylvestris* L. distribution throughout the Lateglacial and Holocene is analysed using machine learning techniques (Random Forest) and environmental variables.

Old scenarios which set off during the Last Glacial Maximum (21000 BP and 6000 BP PIMP scenarios) as well as future predictions (IPCC A1, A2, B1, B2 scenarios) have been used.

Our results show an area reduction during the LGM and a later spread agreeing with the recovery initiated 6000 years ago. The presence of isolated populations at low altitudes could show the migratory paths followed by the Northeuropean taxa.

Even the less drastic future scenarios illustrate a rather fast decrease in the Iberian *Pinus sylvestris* area. Its distribution is restricted to the Northern mountains at the most elevated cotes disappearing from the Southern and Centre montane areas.

Pinus sylvestris reaches at the Iberian Peninsula its Southern limit. This work shows the global warming effect over this kind of taxa and denotes the relevance of this tool for land conservation and management.

P2177. Herb-chronology - a handle on growth responses of perennial forbs to changing environments

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Analysis of annual rings in the roots of perennial forbs (herb-chronology) may be used to reconstruct fluctuations in growth due to environmental variation. Here we ask whether and how growth trajectories of forbs change along strong environmental gradients at various scales and whether climatic fluctuations impact plant growth on top of such responses. Both forbs growing in alpine snowbank gradients and forbs occurring over pronounced elevational gradients showed considerable changes in growth patterns during their life that may be interpreted as adjustments of individual life histories. In addition, a synchronous impact of strong, large-scale climatic events on growth increments in the roots could be confirmed for a wide range of forb species in various habitats of the Swiss Alps and the continental US. Our results suggest that herb-chronology is a valuable approach to investigate how gradual environmental change (e.g. global change) and a higher frequency of climatic extremes may affect the growth behaviour and competitiveness in perennial forbs.

P2178. Climate Effects on Steppe Ecosystems and Land-use in the southern Russia

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Steppe zone in Russia has been extensively used in agriculture for 200 years. Under conditions of destabilized environment and modern climate fluctuations (increase of precipitation) new hydromorphic ecosystems are formed on the watersheds and their slopes. Increase of precipitation by 30% (comparing with long-term annual amount) and warm winters occurrence lead to abrupt jump of the waterlogged areas. Though the changes are not statistically significant (poor trends) it leads to tremendous changes of environment- these ecosystems are characterized by surplus stored moisture and high salinization of chernozem soils. Therefore these high productive soils are degrading and are replaced by fallow lands. Weed communities (reed, thistle, ambrosia, etc.) are formed. They don't maintain steppe biodiversity and are considered as sources of biological pollution. It's necessary to carry out monitoring of the hydromorphic ecosystems in order to make prognosis of their state and to manage the environment properly.

P2179. Impact of climate change on the coastal flora of Germany

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To evaluate the climatic sensibility of vascular plant species occurring at the German coast (North Sea and Baltic Sea), climate envelopes has been estimated for 223 taxa. Correlation of distribution patterns and climatic data allows a prediction of area dislocations caused by climatic change for particular plant taxa. Under a high scenario (warming of 2.5 K up to 2050), the climate envelopes for about 30% of the species in the model show either an expansion or a reduction within the area of the German coast. The warming of 2.5 K equals a spatial shift of about 400-600 km. The speed, at which coastal species will really react to climate change depends mainly on characteristics of their life cycle, seed dispersal as availability and fragmentation of suitable habitats, too. Dispersal by sea current, tidal movement, and wind drift along the coastline will happen faster than for species of mainland biotopes, as potential dispersal routes here are not fragmented as they are in cultivated landscapes. Hence, the German coast is an appropriate area to establish monitoring programs with the aim to detect species shifts caused by climate change and to improve the predictive models.

P2180. Fluctuations of *Festuca varia* grassland (Teberda Reserve, the Northwestern Caucasus, Russia) over 18 years

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Dynamic of an alpine plant community dominated by tussock grasses *Festuca varia* Haenke and *Nardus stricta* L. was studied at the Mt. Malaya Khatipara (2750 m a.s.l.) in the Teberda Reserve, the Northwestern Caucasus. During the period 1987 - 2004 we counted shoot number of all plant species on 40 permanent plots 25x25 cm (total area 2,5 m²). We selected 22 abundant species for detail analysis from total number of 43 recorded over 18 years. We recognized three groups of species according to their dynamic behavior. Shoot number of *Calamagrostis arundinacea*, *Campanula collina* and *Galium verum* increased significantly ($p < 0,01$) over the observation period. Abundance of these species was positively correlated with maximal winter temperatures of the previous year. Shoot number of *Campanula tridentata*, *Carex atrata*, *Matricaria caucasica*, *Minuartia aizoides* and *Viola altaica* decreased significantly during the period of observation. Other species including main dominant *Festuca varia* did not show directional changes. Their number was correlated with various meteorological parameters of the previous and current years.

P2181. Phenological and physiological responses to drought of *Pinus halepensis* in a climate transition zone.

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Relatively rapid climate change will induce unknown adjustments in ecosystem function and distribution that depend on inherent abilities to respond to the new conditions. Of particular importance in many regions is the effect of increasing drought. We explore this issue by taking advantage of a *Pinus halepensis* afforestation site in a Mediterranean - Semi-arid climate transition zone, where summer drought of up to 8 months spans most of the annual foliage growth period. Hence survival depends on meeting both the phenological demands and physiological stress responses. From measurements under the natural drought and summer irrigated conditions we show that the trees have both fixed and flexible phenological responses, and up-regulate drought and damage avoiding strategies to maintain consistent, although reduced, foliage growth. Fully functional new foliage at the start of the wet season then enables optimal carbon gain through the favourable period. In addition, a strong down-regulation of summer time respiration minimizes carbon losses, and the forest is able to sustain a level of productivity that is of a similar order as ecosystems of more mesic regions.

P2182. Long-term reaction of four alpine communities for ecological conditions change: results of 16-year transplanting experiment

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This work is devoted to investigation of relationship between different species and influence of abiotic factors on development of alpine communities. The experiment was carried out in 1989 on Mt. Malaya

Khatipara at 2800 m a.s.l. in the Northwestern Caucasus, Russia. The experiment consisted of reciprocal transplantation between four investigated communities. For 16 years of observation species composition of investigated plots significantly has changed. This included significant changes in shoot amount of several species as well as introduction of species from surrounding communities. The reaction of the same species had appeared unequal in conditions of different treatments, and reaction of different species in the same conditions also was unequal. We conclude that alpine communities include species of different ecological requirements. There are species, for which the ecological optimum coincides with their real habitat, and the species, which with larger success develop in other ecological conditions.

P2183. Medicinal and aromatic plants of mountainous grasslands in Serbia

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In order to estimate biodiversity and utilization potential of grasslands of the hilly-mountainous regions in Serbia, it was analyzed the presence of medicinal flora in 12 widespread communities of the classes *Festuco-Seslerietea*, *Nardo-Callunetea* and *Juncetea trifidi*. In these plant associations 88 medicinal and aromatic plants from 58 genera and 28 families were recorded, which accounts to 44.44% of the total number of medicinal plants officially registered in Serbia. The special attention was paid to endangered, vulnerable and locally rare plants, as well as those whose irregular exploitation may cause risk for survival of their populations. Among them the following species may be designated: *Veratrum album*, *Arctostaphylos uva ursi*, *Vaccinium vitis-idaea*, *Satureja* sp., *Orchis* sp., *Micromeria* sp., etc. High floristic diversity of medicinal plants of analyzed meadow and pasture communities indicates the opportunity for alternative managing of such natural resources other than traditional agriculture, but concomitantly warn for necessity of sustainable exploitation and protection of particular areas where rare medicinal plants grow.

P2184. Synanthropisation versus changes in vertical distribution of vascular plants in Slovakia (Central Europe)

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The knowledge base of actual distribution area provides opportunity for evaluation of long-time changes in distribution and thus consequently provides opportunity for supposition of next changes in vegetation structure and potential endanger of worthwhile natural ecosystems by synanthropic and invasive species' extension. Besides the progressive, gradual extension, there can be often observed the spontaneous "jump-extension". Vertical distribution of vascular plants was observed by GPS equipment in selected Slovak mountains during 2000-2004. According to the type of biotope and main way of distribution we recognize the species on the road-sides, in settlements, at down-hill tracks and mountain peaks. We observed new altitudinal maxima in Slovak flora and more synanthropic plant species on the vertical border of their distribution in West Carpathians. Spreading of species in the higher altitude is influenced by tourism, transport development and gradual climate change, too.

P2185. Contributions regarding the study of the *Juncetea trifidi* Klika et Hadac 1944 Class in the upper basin of the Luncavat River (the County of Valcea)

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The territory under research is located along the upper course of the Luncavat River and it covers an area of about 450 square kilometres. From the geo-morphological point of view, the upper basin of the river includes two, well-defined levels of relief: the mountain region, belonging to the Capatanii Mountains, in the Southern Carpathians, and the Horezu SubCarpathian Depression, which is part of the SubCarpathian region of Oltenia. The present paper aims at presenting the associations of the *Juncetea trifidi* Klika et Hadac 1944 Class, met in the upper basin

of the Luncavat River. In the territory under research, there were identified three vegetal associations as being part of this class: *Primulo-Caricetum curvulae* Br.-Bl. 1926 em Oberd. 1957, *Oreochloo-Juncetum trifidi* Szafer et al. 1927 and *Potentillo chrysocraspedae-Festucetum airoidis* Boscaiu 1971 (Syn. *Festucetum supinae* Domin 1933).

The intensive grazing has a negative impact upon the vegetation in the upper basin of the Luncavat River, because of the decrease in the vegetal biomass and in the number of species with a fodder value.

P2186. Forest expansion patterns of natural forest-grassland mosaic in southern Brazil.

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In general, the climatic conditions in southern Brazil are suitable for forests, but actual vegetation consists of a mosaic of grassland and forest. The aim of this study is to evaluate invasion patterns of woody species into grassland in Porto Alegre, Brazil, comparing possible forest expansion into grassland through encroachment of isolated shrubs within the grassland, shifting of the forest edge and establishment of Isolated Woody Patches (IWPs). We focused on floristic and structural relations under different environmental conditions. The cluster and ordination analysis show an increase in structural complexity from small to large IWPs. The structuring and expansion process lead to an increase in diversity, and are strongly related to IWP size, source distance and isolation level. Two different main patterns types were identified: first, the early IWP, with most of the species dispersed by animals and, as it get larger and structurally more complex, it become more similar to the forest edge; the other type is characterized by gradual colonization of forest woody species into the open grassland, where they exist together with grassland woody species.

P2187. Bio-Ecological Study of Some Low Grasses of Flora of Georgia

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The aim of the study was to investigate bio-ecological and decorative characteristics of some low grasses of wild Georgian flora; to assess the possibility of application of these characteristics.

We studied 21 families, 78 genera and 210 species of low grasses which have specific shape and decorative properties.

We defined eco-morphological types of investigated low grasses: hydrophytes, heliophytes, heterophytes, xerophytes, halophytes, drought resistant, frost resistant, light demander and shade tolerant grasses. We also defined their phenological spectrum and decorative properties.

Our study confirms that low grasses developed special vegetative organs during the process of the evolution. These organs, such as root, stolon, rhizome, buble and tuber are considered as reproductive organs. They have common functions but differ in their morphological properties, which was proven by additionally performed morphogenesis on the studied species.

P2188. Populations diversity in Neotropical savannas

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Approximately 25 % species in northern South American savannas inhabit a wide range of sites with contrasting water and nutrient availability. As land use changes and biological invasions compromise savanna's persistence and function, it is important to evaluate intraspecific diversity to select populations suited for conservation and restoration.

Venezuelan savannas were censused and ecophysiological traits of species from contrasting functional groups were determined. Isozyme diversity was measured in the prominent grass *Trachypogon plumosus*.

In all species, specific leaf area (SLA), N, P, ¹³C content and assimilation rate varied clinally between local populations. SLA decreased towards the driest sites whereas N and P leaf content was highest in the fertile sites. Isozyme diversity in *T. plumosus* was moderately high (H = 0.291 - 0.431) and 28% of it was interpopulational suggesting local adaptations which contribute to regional diversity. If quantitative traits and isozyme differences of other species mimic those of *T. plumosus*, then the diversity of

northern South American savannas should be much higher than that represented by species richness.

P2189. Important Plant Areas of Central and Eastern Europe - priority sites for plant conservation recognised.

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Important Plant Areas, the best sites for wild plant and fungi conservation, have recently been identified in 7 countries of Central and Eastern Europe (Belarus, Czech Republic, Estonia, Romania, Slovakia, Slovenia), according to consistent scientific criteria - threatened species, botanical richness, and threatened habitats. Preliminary results have been reported from countries in South East Europe (Bulgaria, Croatia, Macedonia (FYR) and Serbia and Montenegro).

Important Plant Areas (IPAs) are natural or semi-natural sites exhibiting exceptional botanical richness and/or supporting an outstanding assemblage of rare, threatened and/or endemic plant species and/or vegetation of high botanical value.

Hundreds of specialists from academic institutions, state conservation agencies, and NGOs identified almost 800 IPAs for higher plants, fungi, mosses, lichens and algae. Conservation status, threats, land use and many other data were collected about IPAs to provide essential information for conservation management and planning. The results of the project are published in a comprehensive overview of plant conservation.

P2190. Floristic and phytocoenological analysis of the meadow community *Agrostietum vulgare* Pavl.1955 in east Serbia

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Communities of the *Agrostietum vulgare* type have being formed after cut off of the beech woods developed in the hilly-mountainous regions in east Serbia. Floristic and phytocoenological analysis of this association was performed on the basis of a survey of its 19 stands spread on the Mt. Stol in east Serbia. Besides the edificatory species, i.e. *Agrostis vulgaris* L., whose values of abundance and coverage were the highest (7-8 according to the scale of Westhof and van der Maarel), the following species among the total of the 125 recorded, were assigned as characteristic: *Briza media* L., *Festuca rubra* L., *F. ovina* L., *F. valessiaca* Schl., *Filipendula hexapetala* L. *Achillea millefolium* L., *Trifolium montanum* L., *T.alpestre* L. *Stellaria graminea* L., *Rhinanthus rumelicus* Vel., *Hypochoeris maculata* L., *Holcus lanatus* L., *Trisetum flavescens* (L.) P.B., and *Thymus pulegioides* L.

By use of numerical classification (Generalized Eucliden Distance) and ordination (CA analisis) it was showed that there are three coenotic blocks, indicating the differentiation of this community due to variability in habitat conditions, primarily climate and soil characteristics.

P2191. Vegetation patterns across the timberline ecotone in the eastern Pyrenees

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The *Pinus uncinata* timberline has been analysed in 12 plots representative of the diversity of conditions (topography, bedrock type, land use) in the eastern Pyrenees. In each plot -a rectangle set along the altitude gradient and including from close forest till typical alpine pasture- three data sets were carefully recorded: tree structure (including explicit individual location), vegetation (small, intensive plant relevés along the rectangle) and substrate particularities (plant cover, bare rocky outcrops, slope).

The elaboration of our data illustrates a general upslope increase of diversity at small scale, which becomes the most pronounced not exactly at the timberline, but higher, coinciding with the decline of sub-shrubs. Site conditions (bedrock type, skeletal soils, 'natural' vs. anthropic timberlines) are related to species richness at bigger scale, definition of particular ecotone communities, or other particular trends. Upwards timberline displacement cannot be forecasted as general; where previewed, it would mean some reduction in the area of alpine pastures against more rocky surfaces.

P2192. Diversity of grassland flora and vegetation after several decades of land use changesM. Janisova¹, E. Uhliarova², K. Ujhazy³;¹Institute of Botany, Bratislava, Slovakia, ²Faculty of Science, Matej Bel University, Banska Bystrica, Slovakia, ³Faculty of Forestry, Technical University, Zvolen, Slovakia.

The detailed study of flora and vegetation was performed during the years 1994-2004 in the Protected Landscape Area and Biosphere Reserve Polana (Central Slovakia). All types of grassland ecosystems were included and compared as to the diversity of vascular plants and their communities. Phytosociological approach of the Zurrich-Montpelier school was used for the analysis of vegetation. The studied region, originally managed in the traditional manner, was subject to dramatic changes in land use since the middle of the last century. Many grasslands were abandoned or converted to the extensive use. Still, in some areas the traditional grassland management has maintained. A comprehensive analysis of distribution of both individual taxa and syntaxa supported by the analysis of relevant environmental factors was used to reveal links between the management types and the grassland diversity characteristics. This work was supported by Science and Technology Assistance Agency under the contract No. APVT-51-015804, and by VEGA 1/0126/03.

P2193. A study of Kotayk's (Republic of Armenia) present vegetation compared with the data of the passport system of natural fodder lands in 1946

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The study of the modern state of Kotayk's fodder lands vegetation, taking into account the relevant data of the passport system of natural fodder lands conducted in 1946, shows that the edifiers of the natural vegetation have changed, for the last 55 years. Thus, if in 1946 edifiers were *Stipa capillata* L. and *Festuca valesiaca* Gaud., now they have been replaced by *Festuca valesiaca*, *Taeniatherum crinitum* (Schreb.) Nevski and *Artemisia fragrans* Willd.

Due to the irrational and most intensive use of those pastures, cereals and leguminous plants formations were replaced by diverse and ephemeral grasses in which weeds and other inedible plants have become prominent. Considering the total aridity of the Republic's climate, the early pasturage and excess grazing have led to emergence of the transition semi-desert vegetable grounds with *Artemisia fragrans* and *A. austriaca* Jacq. and as a result the fodder lands productivity is reduced twice.

P2194. Recent vegetation dynamics of "classical karst" (Slovenia): from habitat to landscape levelM. Kaligarič¹, M. Culiberg²;¹Biology Dept., University of Maribor, Slovenia, ²Biological Institute Jovan Hadzi ZRC SAZU, Ljubljana, Slovenia.

Cartographic material and habitat mapping were applied to reconstruct the temporal dynamics of grassland vegetation of the "classical karst" (Slovenia) for last 250 years. Pollen data were used in order to reconstruct the vegetation history of secondary grasslands since late-Holocene till Middle Ages. The 250 years old maps shows that grasslands covered 81 % of the "classical karst" pilot area of total surface of 665.8 km². Only 25 % of the same territory was covered with grasslands in 2003. There was also substantial increase of fields, vineyards and orchards - from 1.5 to 4 % and settlements from 0.5 % to 4 %. On a sub-pilot area of 14 km² habitat mapping in 2003 shows that less than a half of the grasslands on the sub-pilot area are still typical meadows or pastures, without signs of scrub/forest or tall-herb progression and only a few hectares are still mown or grazed. The "grassland period" of the "classical karst", associated with the beginning of civilisation and strong human influence, was neither a linear process nor a stable stage. It was a very dynamic period, with permanent species replacement due to different mode, extent and intensity of land-use.

P2195. Predicting grassland habitats distribution upon different scenarios of landscape changes

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Natural and semi-natural grassland habitats represent a specific feature of the Poloniny National Park (Slovakia) with the great

biodiversity values. However, agricultural decline and consequent landscape changes in this region affect grassland habitats significantly. We compared grassland area and distribution in the study area in years 1949, 1987, and 2003. Significant decrease both in grassland area and structure of distribution was recorded. We attempt to predict future distribution of grassland habitat types based on different scenarios of the landscape development. The scenarios derived from different possible agricultural policies in the study area. Combination of habitat suitability modelling and landscape change modelling was used. The total area and distribution structure of respective grassland types in year 2030 upon different scenarios represent the main results of the work.

P2196. Retrogressive dynamics in the *Poion alpinae* pastures from the south-eastern Prealps (the Brocon Pass, Italy)D. Gafta¹, F. Pedrotti²;¹Babes-Bolyai University, Cluj-Napoca, Romania, ²University of Camerino, Camerino, Italy.

Patches of *Trollius europaeus* assemblages (T) and especially, *Deschampsia cespitosa*-dominated (D) communities are spreading within *Poion alpinae* pastures (P). The ecological gradients underlying these dynamic processes were inferred by indicator species analysis performed on T, D and P relevés groups, and by species response curves along the first two DCA axes. A decline in plant diversity and an increasing cover of nitrophilous species occur along the first axis, from P to T and D communities. The differentiation between T and D along the second axis is due to a decreasing cover of basophilous species accompanied by an increment of the acidophilous ones in the latter community. Whereas T forbs-dominated communities occur exclusively on dung deposits, the D ones lie on flat ridges or gentle slopes often trampled by livestock. Upper soil compaction and the accumulation of a cellulose-rich litter below the *Deschampsia* tussocks are probably responsible for the high moisture fluctuation and bases depletion. The return to a traditional use of *Poion alpinae* grasslands (alternating mowing and grazing) might stop the observed retrogressive processes.

P2197. The seedling recruitment and survival under different management regimes

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Absence of suitable microsites is assumed to be a reason for loss of species from grasslands during degradation process. Therefore, one important aim of restoration management is to enhance the regeneration. Studies have shown that mowing and grazing promotes seedling recruitment. However these conclusions are based on single records and it is not known, whether the increased recruitment really results in higher number of established individuals.

The aim of this study is to evaluate the effect of different management regimes on seedling establishment. Abandoned pasture in Beskydy (Czech Republic), where the sheep grazing was reintroduced, was chosen as a study site. Eight blocks of permanent plots: grazed, mown and abandoned were set up for monitoring. The survival of dicots was recorded for 2 years.

The number and species composition of seedlings changed during the study period and it varied between the treatments. Overall the mortality of seedlings was very high. This shows that conclusions based on one time census only can be quite misleading. Long-term studies are needed before we conclude that the particular management can really enhance establishment.

P2198. Phytocoenotic and biogeochemical peculiarities of serpentinite steppes of Southern Ural

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The geochemical features of steppes landscapes are predetermined by all complex of the natural factors, mainly by lithogenic basis, which is conservative block of natural geosystems. One of the most interesting types of steppes of Southern Ural with phytocoenotic and biogeochemical positions are serpentinite steppes, which originality in arid climate is caused by direct influence of lithogenic basis. Petrophyt variants of steppes with domination of edifier *Festuca valesiaca* and original species-indicators are formed at close bedding of serpentinite rocks. Halophyte variants of steppes with domination

of the same edifier and halophyte herbs (indicator *Orostachys thyrsiflora*) are formed at development of weathering crust of various genesis. Lithogenic basis predetermines also natural contents of chemical elements in plants. Ni, Co, Cr, Ti prevail in chemical structure of plants of serpentinite steppes. The concentration of accompanying elements depends on variant of steppes. The study of serpentinite flora is interesting as in evolutionary aspect, as in aspect of species tolerance to the extreme geochemical factors.

P2199. Dynamics of deserted ecosystems of Gobustan in Azerbaijan

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Deserted ecosystems of Gobustan, adjoining to Apsheron represent interest in connection with influence of technogenesis, proceeding on a background of actable natural ecological conditions. Formation new and serial groupings, caused by a course natural exoecogenetical changes ecotone and technogene desertification caused by the anthropogenous factor have also been observed. Species of meadow vegetations penetrate in hydrohalophyte communities or on sites of xerophytes pettyhilly deserts. Return processes have been marked also. Central Asian, Mediterranean, sometimes Transcaucasian floristic elements, in particular *Tamarix ramosissima*, *Halocnemum strobilaceum*, *Kalidium caspicum*, *Salsola nodulosa* and others are most active. The steppe kind *Colpodium humile* is for the present rare on degrading saline soils. Technogene character of human impact causes pollution and infringement of granulometrical structure of ground, change of a microrelief borrows the special place in this question. As consequence vegetative communities of deserts start to degrade that in general conducts to desertification of its significant territories.

P2200. The Valley of Kamienna River as a refuge of relic xerothermic vegetation in SE Poland

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In Poland xerothermic plants have three main centers of their concentration: in the lower parts of the Oder and Vistula rivers and uplands of southern Poland. The Kamienna river valley (left side tributary of the Vistula) lying at the northern edge of Malopolska Upland is a significant refuge of relic xerothermic vegetation. In its territory occur some rare species in Poland, which are inserted in red data books and lists considered as an endangered. Some examples of them: *Achillea setacea*, *Adonis vernalis*, *Carex pediformis*, *C. supina*, *Cerasus fruticosa*, *Festuca makutrensis*, *Linum catharticum*, *Orobanchella alba*, *O. picridis*, *Orthocentrus lutea*, *Senecio integrifolius*, *Stipa capillata*, *S. joannis*, *S. pulcherrima*. The most of them have pontic-pannonian or subirano-turanian ranges. The way of migration into the Kamienna valley followed by the Vistula river. Two of mentioned species eg. *Carex pediformis* and *Festuca makutrensis* have in Poland few existing sites; that make a part of their local northern limit. This territory because of its floristic unique ought to be included to the list of protected areas in Europe scale.

P2201. Switch from facilitation into competition in an arid rangeland

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Plant interactions were investigated between perennial *Artemisia aucheri* and annual *Bromus tectorum* in an arid rangeland. Temperature, luminance, and soil relative humidity were measured as paired samples from *Artemisia*-understory and neighbouring open space. Regression relationships were investigated between the microclimatic factors and *Bromus* density in understory, or *Artemisia* vigour in overstory. Our findings suggest a cyclic dynamic between the two plants. Accordingly, at the first phase there is an open space in which *Artemisia* seedlings can establish during wet years. Lower light intensity, soil temperature and evapotranspiration facilitates establishment of *Bromus* under *Artemisia* canopy. On the other hand with increasing *Bromus* density in understory, plant interactions switches from facilitation into competition which leads to lower vigour and eventual death of *Artemisia*. Water or wind erosion removes litter and accumulated soil under the dead *Artemisia*, leading to unfavourable conditions

and disappearance of *Bromus*. The cyclic dynamic may also be mediated by allelopathic effects of *Artemisia* on *Bromus*.

P2202. Modification of herbaceous floodland vegetation depending on water regime changes at Gemenc, Middle-Danube-valley, Hungary

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Between 2002 and 2004 vegetation mapping and descriptions were made four times at Nagy-Gyékényes. In 2002, after the first survey, a serious flood and a long, extraordinarily high-water period occurred, followed by a longer drought in 2003. The drastically different water regime states row each other had strong effects on vegetation. For the analysis ecological indexes and social behaviour types of species, and vegetation maps were used. The collected data simply and clearly show that the herbaceous vegetation of the floodland reacts fast and sensitively to changes of accessible water. At first, because of the constant water coverage the species richness of the dominantly alluvial meadow vegetation, patterned based on microrelief, reduced and the dominant role was assumed by hygrophilous and aquatic elements. The less water adherent vegetation types persisted only at the highest levels. In the following drought period an open herb layer remained which was soon filled up by fast propagating, disturbance tolerant weed and invasive species, furthermore the pattern became much homogenous, that all signify the vulnerability of the habitat type.

P2203. Steppe vegetation of the forest-steppe zone of Ukraine

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The steppe vegetation of the Forest-Steppe zone of Ukraine are diverse and endangered, now occupying less than 1% of Ukraine's area (natural percentage was ca. 30%). They belong to the class *Festuco-Brometea* Br.-Bl. et R.Tx. 1943, order *Festucetalia valesiacae* Br.-Bl. et R.Tx. 1943, and are represented by 5 alliances. Communities of the alliance *Astragalo-Stipion* Knapp 1944 unite typical steppes with domination of xerophytic herbaceous vegetation. Communities of the alliance *Fragario viridis-Trifolion montani* Korotchenko & Didukh 1997 represent the zonal meadow-steppe vegetation. The alliance *Cirsio-Brachypodium pinnati* Hadac et Klika 1944 em. Krausch 1961 contains the most mesophytic extrazonal meadow steppe communities, which are climatically and edaphically defined. Communities of the alliance *Festucion valesiacae* Klika 1931 are formed due to anthropogenic transformation of the plant cover and represent various stages of overgrazing. Communities of the *Artemisia marschalliani-Elytrigion intermediae* Korotchenko & Didukh 1997 alliance are formed on steep slopes with denuded soils and a mosaic herbaceous plant cover.

P2204. Taiga-steppe communities of the Baikal region (Eastern Siberia)

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The presence of undergrowth and pine and larch saplings in steppe grass communities is indicative of trends toward a spatial expansion of tree ecobiomorphs in connection with an increase in yearly mean precipitation in the Prebaikalia for the last 30 years. There has been a reduction of areas occupied by steppe communities, and an active penetration of mesophytes into steppe coenoses. There is a spatial expansion of mosses characteristic for the polydominant light-coniferous - dark-coniferous taiga. The structure of the taiga-steppe communities may be represented as a system where the coenoses consist of the tree layer dominated by pine or larch. The soil cover includes the species composition of different variations, and often complexes of mosses and xerophytes. The taiga-steppe communities are characterized by the one- or two-age composition of tree species, and by the polydominance of the shrub zone and grass cover depending on the type of coenose habitat.

P2205. Testing for belowground resource partitioning in experimental grassland communities

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Niche differentiation due to resource partitioning is a possible explanation for positive diversity-productivity relationships in terrestrial plant communities. However, experimental evidence for resource partitioning among different plant species is scarce. In addition, it is far less known whether niche separation can change along gradients of increasing species diversity. In a temperate grassland field with experimental communities of different plant species richness we tested for resource partitioning of soil nitrogen between plant species in monocultures, 3-species and 6-species mixtures. Nitrogen uptake by plants was tracked using ¹⁵N-tracer solutions and determination of the ¹⁵N/¹⁴N ratio in aboveground plant material after 48h of incubation. Six different treatment combinations consisting of three different chemical forms of ¹⁵N-labelled nitrogen (nitrate, ammonium and glycine) injected at 2 depths (3 and 12cm) were applied to each community in a split plot design.

We present species-specific nitrogen uptake patterns and discuss how these are related to niche separation along the existent gradient of plant species richness.

P2206. Contributions regarding the study of the Juncetea trifidi Klika et Hadac 1944 Class in the upper basin of the Luncavat River (the County of Valcea)

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The territory under research is located along the upper course of the Luncavat River and it covers an area of about 450 square kilometres. From the geo-morphological point of view, the upper basin of the river includes two, well-defined levels of relief: the mountain region, belonging to the Capatanii Mountains, in the Southern Carpathians, and the Horezu SubCarpathian Depression, which is part of the SubCarpathian region of Oltenia. The present paper aims at presenting the associations of the Juncetea trifidi Klika et Hadac 1944 Class, met in the upper basin of the Luncavat River. In the territory under research, there were identified three vegetal associations as being part of this class: *Primulo-Caricetum curvulae* Br.-Bl. 1926 em Oberd. 1957, *Oreochloo-Juncetum trifidi* Szafer et al. 1927 and *Potentillo chrysocraspedae-Festucetum airoidis* Boscaiu 1971 (Syn. *Festucetum supinae* Domin 1933).

The intensive grazing has a negative impact upon the vegetation in the upper basin of the Luncavat River, because of the decrease in the vegetal biomass and in the number of species with a fodder value

P2207. The effect of slug grazing on vegetation development and plant species diversity in an experimental grassland

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Generalist herbivores such as slugs have the potential to alter species diversity within vegetation.

We investigated the influence of slugs on plant species diversity in experimental grassland swards in field plots. Half of the plots were grazed by slugs (*Arion lusitanicus*).

Vegetation cover increased in the control plots from 50% in the first year to 90% in the third year. Cover was significantly lower in the slug plots in the first year while there were only small differences between treatments in the third year. Slugs reduced total aboveground biomass by more than 25% in both the first and third years.

Slugs had a negative impact on plant species diversity in the first year. In contrast, plant species diversity after three years was higher in the slug plots than in the controls.

Our results suggest that the effect of slugs upon plant species diversity changes during the course of succession. In the earliest stages slug grazing leads to reduced species diversity because favoured species are eliminated. In closed vegetation slugs may reduce the dominance of the more competitive species. As a consequence, slugs tend to cause an increase in plant species diversity.

P2208. Changes in mosaics of steppe slopes and rocky grasslands at the Balaton-highlands (Transdanubia, Hungary): Aszófo: Öreg-hegy, Balatonfüred: Péter-hegy, Litér: Mogyorós-hegy)

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At three dolomite hills of the Balaton-highlands 50-50 pseudorandom coenological recordings (1x1 m) were carried out on rocky steppe slopes at 50x50 m plots in 2001 and 2003. Based on the classification (Glob. opt.) and ordination of the 300 datasets the followings are noted: The surveyed plots are mosaics of minimum three plant associations: 1. *Chrysopogono-Caricetum humilis* (C-C), 2. *Stipo ericocauli-Festucetum pallentis* (St-F), 3. *Seseli leucospermi-Festucetum pallentis* (Se-F). These associations appear as mosaics and are embedded in each other at variable rates. Clear stands, in the classical coenological sense, can be recorded only by aimed sampling and, at some places, with much smaller sampling units only. Among the three sample plots smaller macroclimatic (decreasing amount of precipitation eastward) and mesoclimatic (decreasing forestation eastward) difference is characteristic. At the western plot which is mosaic with karst shrub-forests it is the more closed (C-C), and at the Eastern plot it is the more opened (St-F) types of datasets that become dominant in the rocky grassland-steppe slope mosaics characterized by variable patch sizes.

P2209. Connection between geobotanical factors, landscape changes and human endangerment on the eastern part of the Dorozsma-Majsaian Sandlands, Hungary

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The making of the actual biotop-map of the Dorozsma-Majsaian Sandlands on 1:25,000 scale started 3 years ago. In this work the connection between geomorphology, soils and vegetation was studied. It resulted that the sandveil-hills covered by humic sand soil are dominated by sandy steppe-fields.

In the depressions a new phenomenon was found: the *Molinia* fens are situated on the northwestern parts (fenhead) while the alcali-sodic vegetation can be found only at the southeastern parts (alcali-sodic foot). The fenheads have carbonated meadow soils or boggy meadow soils while the alcali-sodic feet have carbonated solonchak-solonetz soils.

A landscape scale gradient was also found here. It means that some of the components of the alcali sodic vegetation types disappear westwards. It is also typical that westwards the open sandy grasslands become more common, while on the eastern parts just sandy steppe-fields can be found. The *Molinia* fens are more common in the western and mid-part of the Dorozsma-Majsaian Sandlands.

On a smaller sample area the history of the landscape was studied with the help of the 1st (1764-1787), 2nd (1806-1869) 3rd (1872-1887) military surveys.

P2210. Primary production and biomass of Sabarmati estuary in the gulf of Cambay, Gujarat, India

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The present study focus on the spetial and seasonal pattern of phytoplankton density, chlorophyll -a as a phytoplankton biomass and primary production in relation to some hydrographical parameters were studied for two years at five sites of the Sabarmati estuary, in the Gulf of Cambay, India, for Two years [December 2001 to December 2003.]

The phytoplankton population density varied from 980 to 2730 cells per liter. it was higher during winter season at all the sites and low during monsoon season. The pigment concentration particularly chlorophyll-a as a biomass was observed between 0.60 to 2.45 mg/m³. The maximum values were observed during winter, while lower during monsoon. The gross primary production varied between 0.0031 to 0.072 mg. carbon fixed per liter per hour. The fluctuations in some of the environmental characteristics i.e. physicochemical parameters characteristics, minerals and trace metal concentration in water have been discussed with their relative influence on the production of phytoplankton and primary production.

P2211. Apparently well-known diatoms can produce unexpected offspring

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Whatever organisms are used, biomonitoring rests on the reliable recognition of taxa of known ecological ranges and tolerances. Diatoms are routinely used to assess river trophic status, with some taxa designated as organic pollution indicators, e.g. *Gomphonema parvulum* and many *Nitzschia* spp. Although there are exceptions, many diatoms undergo marked size reduction over their life cycle, and the extremes of the size range may appear unrelated. Observation of sexual reproduction is required to link the different morphs. Sexually reproducing clones of two *G. parvulum* varieties in culture produced offspring identified as *G. gracile* and *G. hebridense*, neither of which are considered pollution tolerant. Similarly, sexual reproduction in a small *Nitzschia* (*N. frustulum* complex) also produced markedly different offspring. The implications of these findings for diatom taxonomy and for the use of such species as bioindicators will be discussed.

P2212. Trophic Status Of The Lakes Of Bhopal, India

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The present paper deals with an assesment of trophic status of the lakes of Bhopal by using Nagaard's compound quotient .There are a number of water bodies in Bhopal , the capital city of Madhya-Pradesh .The most famous among them are the twin lakes called the "Upper Lake" and the "Lower Lake " . The Upper Lake is the main source of drinking water for the city and is partly influenced by human activities .While the Lower Lake ,which occupies the central position in a thickly urbanized locality,has greater man-made disturbances .Both the lakes are being polluted by the domestic sewage and other recreational activities .As a result there is a gradual deterioration of water quality which in result affects the composition of algal flora.

Monthly algal collection were made from each lakes and water samples were analysed for different physio-chemical parameters.A total number of 603 taxa belonging to 158 genera have been identified .Out of the 603 taxa described ,27 have been considered as new record for India .These were grouped according to classes to calculate Nagaard's compound quotient to show the degree of eutrophication.

P2213. The microphytobenthos of Kyiv site of Kaniv reservoir

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The microphytobenthos structure of Kyiv site of Kaniv reservoir and in particular the effect of daily mode of operations the Kyiv hydroelectric power station to structure and primary production of microphytobenthos have been investigated. Sharp reduction of quantitative indexes of algae and change in dominant complex structure after achievement of maximum water level was established.

The values of gross primary production and destruction in benthos were shown to fluctuate widely under effect of many interdependent and interaction factors, mainly hydrological and light modes, depth, type of soil, structure of the communities of thicket of aquatic macrophytes. Maximum values of primary production have been registered under stable hydrological conditions. It was ascertained that the values of gross and net primary production both on sites without thicket of macrophytes and on sites with thicket, decrease under influence of the raising of water line. The parameters of destruction in benthos on sites without thicket considerably increased, probably because of addition of chemical oxidation of the regenerated forms of chemical compounds of bottom sediments.

P2214. Biodiversity Study Of Cyanophyceae Algae Of Haranbari Dam And Mausam River Of Maharashtra (India)

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Taxonomical studies of algae from various aquatic habitats were studied extensively in India but very few workers have paid attention on these aspects in Maharashtra state of India. To fulfill the lacuna in this field the present investigation was carried out by selecting Haranbari dam and Mausam river from Baglan region of Nashik district of Maharashtra state. In present investigation bio

diversity study of Cyanophyceae algae was made during July 1998 to June 2000 , Eighty three algal taxa belonging to twenty five genera Cyanophyceae were recorded from Haranbari dam and Mausam river from Baglan region of Nashik district of Maharashtra state. In present study the class Cyanophyceae of algae is mainly represented by the species of *Microcystis*, *Chroococcus*, *Gleocapsa*, *Aphanocapsa*, *Synechococcus*, *Gomphosphaeria*, *Merismopedia*, *Johannesbaptistia*, *Hydrococcus*, *Arthrospira*, *Spirulina*, *Oscillatoria*, *Trichodesmium*, *Lyngbya*, *Nostoc*, *Schizothrix*, *Anabaena*, *Pseudoanabaena*, *Raphidiopsis*, *Microchaete*, *Homeothrix*, *Calothrix*, *Rivularia* and *Gleotrichia*. Twenty two species of *Oscillatoria* were recorded in present study.

P2215. Some Epipellic and Epiphytic Algae from Jajrood River Iran

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Diatomes are important part of algal assemblages in aquatic ecosystem . Both Epipellic and Epiphytic flora are major constituents of the algal assemblages . There algae are base of food chain in aquatic ecosystem . The survey was conducted on jajrood river . Epipellic and Epiphytic samples were gathered on monthly intervals from June 2004 through April 2005 . In this study 25 Genera were identified .*Cymbella* with 13 species showed high species diversity.

In epilithic samples *Diatoma* with 8 species was abundant from identified species .17species was observed in epipellic samples and 19 in epiphytic. 66 species were common in both samples .some more encountered species of Diatomes were: *Cymbella* ,*Diatoma*,*Navicula*,*Nitzschia*,*Cocconeis*,*Gomphonema*, *Cymatopleura*,*Didymosphenia*,*Melosira*,*Stephanodiscus*,*Amphora*, *Synedra*,*Hannaea*,*Tetracyclus*,*Achnanthes*,*Surirella*,*Achnantheidium*, *Peronia*.

P2216. The influence of air pollution on the cell size of Xanthoria parietina phycobiont

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Lichens are widely used as bioindicators of pollution level. Phycobiont of lichens is probably more sensitive to pollution than mycobiont.

Lichens of 18 sites in Moscow and Moscow region were studied. *Xanthoria parietina* was found on 16 sites. The phycobiont of *X. parietina* is the green alga *Trebouxia*. The lichen was found on 10 of examined 17 tree species.

Cell diameter of phycobiont ranged from 6.25-16 μ , depending on level of pollution and substrate type. Phycobiont diameter in lichens collected on an unpolluted site did not depend on substrate type.

The smallest cells of phycobiont were in samples of lichens collected on sites with high local level of pollution. Maximum size of phycobiont cells was registered on sites with medium local level of pollution. Medium size of phycobiont cells was registered on sites with low local level of pollution and in lichens of unpolluted site (Tver region).

Size of phycobiont cells can be used as criterion for local level of pollution.

P2217. Dredging impact on the epipellic diatom species composition on mudflat from the Bahía Blanca Estuary (Argentina)

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The dredging in areas of intense harbour activity is cause of considerable alterations in the conformation and dynamics of the biological communities. The aim of this work was to analyse its effects on the epipellic diatom species composition. The study was conducted in muddy tidal flats in Puerto Rosales (38° 53' S 62° 06' O) after 10 days from a dredging, in low tidal conditions. Observations were carried out parallel to the coastline from the dredging site (E3) and other two stations (E2 and E1) 200 m away. Diatom assemblages and density were sampled by the lens tissue method. The nearest station of the dredging zone (E3) showed only fine sediment particles and scarce frustules rest of phytoplanktonic diatoms that indicated the major disturbance due to deposition particles produced by nearby dredging. There were

important taxonomic structural changes in the diatom-dominated and no differences in their density between the rest stations. E2 was dominated by *Surirella minuta* while in the farthest (E1) *Cylindrotheca closterium* and *Gyrosigma spencerii* were the most abundant. The presence of alive benthic diatom populations indicated an active biogenic process.

P2218. Community structure of algae in relation with physical and chemical parameters of the Laguna Piaz, La Libertad, Peru

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The composition and community structure of microalgae was studied in a lake of Peru, which had a strong influence by gold mining activity.

Contamination by arsenic, lead and zinc was found in the main tributary and the initial and medium zone of the lake; it had reported the variability of physical/chemical characteristics of the water during three years and within different seasons between 1999 and 2002.

A total of 55 species have been found; where *Scenedesmus bijuga*, *Oocystis parva*, *Cosmarium quadratum* and *Coelastrum proboscideum* presented the greater occurrences and abundances. The diatoms *Nitzschia palea* and *Navicula pseudobryophila* presented a greater occurrence in the lake and the main tributary. It was the first time that it has been registered in Peru what kind of species lives in this ecosystem.

The ecosystem of lake has a low abundance, low diversity, dominance and a few temporary variations of species.

The cluster and correspondence analysis established the associations of the algae communities, the distribution of species in relationships with the physical/chemical parameters.

P2219. Phytodetection of ambient ozone toxicity in Ukraine

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Ambient ozone toxicity using ozone sensitive (Bel-W3) cultivars of tobacco (*Nicotiana tabacum*) and clover (*Trifolium subterraneum* cv. Geraldton) was assessed. Test-plants cultivated in ozone-free air were exposed in the field (Natl. Bot. Garden, Kyiv, Ukraine) during the summers of 1995 and 2004. The ambient ozone concentrations were continuously monitored by UV ozone analyzer and the moving average of the short term AOT40 were calculated. The antioxidants substances EDU, quercetin and catechin, the fungicide agrochemical "Topaz" (containing 10% of penconazol), as well as water extract from leaves of marigold (*Tagetes patula*) were applied as foliar sprays. The results obtained shows that ambient ozone concentrations were high enough to cause visible leaf injury in subterranean clover and tobacco. The short term AOT40 was positively correlated with visible injury. Not only had the well-known EDU protected test-plants against ozone, but also other applied antioxidants.

P2220. Ozone injury and antioxidative substances in *Trifolium* and *Centaurea*.

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Ozone-sensitive (NC-S clone) and resistant (NC-R clone) plants of white clover (*Trifolium repens*) and brown knapweed (*Centaurea jacea*) have been used in an open air experiment to analyse the development of visual ozone injuries and antioxidant concentrations. Visible injury assessments and harvests were performed as indicated in the protocol of UN-ECE ICP-Vegetation. Between August and October 2004, leaf material of different ages with and without visible ozone injuries was collected for chemical analysis of ascorbic acid, total phenolics and antioxidative capacity. Ascorbic acid (AA) concentrations of young *Centaurea* leaves (1 week) were about 100 % higher than in old ones (10 weeks). Ozone-injured leaves were by 25 % lower in AA than healthy ones. Age differences (1 vs. 4 weeks) in AA concentrations of clover leaves were mostly <10 %. Leaves of the resistant NC-R clover clone had about 50-60 % higher AA concentrations than the sensitive NC-S clone. Additionally, in small open-top chambers an ozone-fumigation experiment was performed with clover plants.

P2221. Ozone alters the relative allocation of resources to vegetative and reproductive growth in European wild plant species

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Little is known about ozone (O₃) effects on species from semi-natural vegetation. For wild species with different ecological strategies traits that relate most directly to ecological fitness (e.g. reproduction, resource allocation) are more relevant for assessing (O₃) effects than e.g. visible symptoms. Experiments with more than 30 common plant species with different life-forms and different families were performed to investigate the effects of (O₃) on plant growth and reproductive development. The relative O₃ sensitivities of the different species in terms of leaf biomass differed from those inferred from total, aboveground biomass and seed or flower production. Although O₃ caused comparable reductions in both vegetative and reproductive biomass in a number of species, in a few species resources were switched to vegetative growth at the expense of reproductive growth. Germinability of seeds was affected such that germination rate was up to 30 % lower in O₃-treated plants compared to control plants.

P2222. Some aspects concerning the reply reactions of some gymnosperms to the action of solid industrial pollutants

L. Ivanescu, M. M. Zamfirache, C. Toma, I. Toma, R. I. Apetrei; University Al. I. Cuza Iasi, Faculty of Biology, Iasi, Romania.

The investigations we made concerning the micromorphology of the foliar surfaces of two gymnosperms species *Pinus sylvestris* and *P. nigra* from Bicaz and Tasca industrial areas (Romania) continue another study (In *Proceedings of the Ecological Protection of the Planet Earth, Xanthi, Greece, 2001*). The electronic microscopy investigations (S.E.M.) made on necrotic and/or chlorosis surfaces of the leaves showed changes of the cuticular surface, caused by the total disappearance of the parallelism between the cuticular prints, the chaotic setting of the stomata and their smaller dimensions, the closing of the ostiols with amorphous wax, which could be an indicator of the "suffering" of the leaf mesophyll in general. So, revealed the role of the solid deposits in the phenomenon of partly or total defoliation of the individuals. Such deposits deactivate important photosynthetic active parts; they hinder the respiration and perspiration and modify the characteristic cuticular relief.

P2223. Characteristics and limiting factors of soil fertility in sand coastal plain vegetation (Restinga Forest) of the litoral area in the state of São Paulo, Brazil

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The sand coastal plain vegetation (Restinga Forest) is a type of ecosystem associated with the Atlantic Forest constituted of mosaics, which occur in areas of great ecological diversity. This work was carried out in several points of Restinga Forest in the litoral coast of the state of São Paulo. One sample was made of 15 subsamples of each area collected in each depth (one in 0 - 5, 5 - 10, 10 - 15, 15 - 20, and another in 0 - 20, 20 - 40, 40 and 60 cm). Soil characteristics analyzed were pH, P, Na, K, Ca, Mg, S, H + Al, Al, B, Cu, Fe, Mn, Zn contents and base saturation, cation exchange capacity and aluminum saturation. The high aluminum saturation and low calcium values to Restinga Forest determine superficial root system, restricting the vegetation growth. The nutritional reserve is mainly in the vegetable biomass. Leaves and root aluminum analyses indicated that does not exist a mechanism of aluminum retention in the root system.

P2224. River vegetation of the Upper Volga Region (Russia)

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Study of aquatic vegetation, in particular its coenotic structure, has a quite long history in Russia. However, the main results are received on water objects with stagnant waters (lakes, reservoirs, oxbows, etc.). The knowledge of very peculiar stream and river vegetation remains weak till now. Investigation of vegetation cover in streams and rivers of the Upper Volga Region are being carried

out since 1994. Vegetation communities of the watercourses are presented by 80 associations from 26 alliances, 14 orders and 10 classes of Braun-Blanquet system. The river vegetation differ by high patchiness and tier structure, diversity of macrophyte groups (macroalgae, liverworts, bryophytes and various groups of vascular plants), penetration of submerged semiaquatic plants into hydrophyte communities, abundance of hybrid, frequently of relict character, plants. The obtained results have shown features of the river vegetation in comparison with vegetation of waterbodies, and its difference from the most well investigated vegetation of rivers of the Western Europe, the latter is predetermined in many respects by more continental and boreal climate of the region. The research is supported by Russian Fund of Basic Researches (project N° 04-04-49814) and Fund of Support to Native Science.

P2225. Nitrogen content among species of pine forests growing along ammonia concentration gradient

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Among nitrogen species in the atmosphere and their deposition on to terrestrial vegetation, ammonia is considered to be the foremost. Estimated by passive diffusion tubes ammonia concentrations along the 22 km transect from the nitrogen fertilizer factory ranged between 0.4-75 µg m⁻³. Along the selected transect there was tendency for more frequent growth of nitrophilic species, (specially *Chelidonium majus*) in Scots pine stands where higher concentrations of ammonia were documented. In the assimilative organs of *Galium album*, *Chelidonium majus*, *Sambucus racemosa*, *Stellaria media*, *Anthriscus sylvestris*, *Rubus idaeus*, *Calamagrostis epigeios* concentration of nitrogen was higher (24-39 µg g⁻¹ d.w.) than in the needles of Scots pine. Concentration of nitrogen in the needles of Scots pine was approaching physiological optimum. Along the transect herbaceous plant species like pines did not differ according to the amount of nitrogen in the leaves, although nitrogen content was among the highest compared to that described in literature. Data obtained show that nitrogen from ammonia deposition is more effectively used by understory species than by pines.

P2226. Phytoindication of the Surface Waters of the Canyon of the River Smotrych (NNP "Podilsky Tovtry")

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¹Kiev National Taras Shevchenko University, Kiev, Ukraine, ²N.G. Kholodnyi Botany Institute of National Academy of Sciences of Ukraine, Kiev, Ukraine.

The research was held on the territory of National Nature Park (NNP) "Podilsky Tovtry", the biggest in Ukraine and the second big as for the territory in Europe.

In course of the research the spreading and ecological peculiarities of the growth of macrophytes-indicators according to hydrological and hydrochemical conditions were established. The index of phytoindication (If) was indicated, which characterizes the condition of waters of the canyon of the river Smotrych as unsatisfactory. The valuations of condition of water ecosystem under of influence of anthropogenic press was given in the comparison of data of phytoindication researches with hydrophysical and hydrochemical parameters.

On the whole phytoindication researches are rather actual especially on the nature protected territories. They give the opportunity to define integral level of pollution as well as general state of water ecosystems. At the same time we have an opportunity to make the discreet control with the help of hydrochemical methods and only in the definite periods of time. And some changes between observations.

P2227. Effects of disturbances on growth dynamic of the seagrasses *Posidonia oceanica* assessed by lepidochronology technique

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University of Calabria, Cosenza, Italy.

The seagrass *Posidonia oceanica* (L.) Delile represents one of the most important primary producers of the Mediterranean coastal zone. *P. oceanica* forms meadows with a multifunctional role: produce and export biomass and energy, host diversified animal and algal community and stabilise coastal sediments protecting from erosion. However, the plant is sensitive to environmental disturbances and useful as bioindicator for environmental conditions: distribution pattern, shoot morphometry and dynamics

of *P. oceanica* meadows are employed to detect an integrated response to disturbances.

In this work we have used the lepidochronology technique (that estimate the age leaf sheath persisting on the rhizomes after the leaf blade fall) as a tool to analyse seagrass dynamic. We analysed two meadows placed along the Dino Island (South Italy) subject to different environmental stress. The results show a decline in the shoot leaf production in *P. oceanica* shoots sampled in the disturbed site vs the preserved one; furthermore the same data provide evidence of the lepidochronology as suitable methods for detecting environmental disturbances.

P2228. Antioxidative responses of duckweed (*Lemna minor* L.) to short term copper exposure

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¹Biotechnical Faculty, Ljubljana, Slovenia, ²Institute of Physical Biology, Grosuplje, Slovenia.

We estimated the potential use of duckweed (*Lemna minor* L.) as an indicator of environmental metal contamination and a biomarker for oxidative stress. Oxidative stress was evaluated during different exposure times and concentrations of the metal salt CuSO₄, using two antioxidant parameters: the activity of antioxidant enzymes (glutathione reductase - GR, catalase - CAT and guaiacol peroxidase - GPOD) and the ratio between oxidized - GSSG and reduced glutathione - GSH. We observed that the enzymes exhibit different sensitivity to copper exposure and that these changes in their activities are time-dependent. GPOD activity of plants exposed to copper was similar to that of controls at low and intermediate concentrations of copper, however, at the highest concentration tested (100 µM Cu) the enzyme activities of exposed plants were higher than that of controls. On the contrary, GR activity was higher than in controls at low copper concentrations, and lower than in controls at high copper concentrations. CAT activity did not differ a lot from control activity, except at 2 µM Cu, where it was higher than that of controls. Potential use of each enzyme test will be discussed.

P2229. The use of bryophytes as monitors of freshwater ecosystems

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Nowadays the aquatic ecosystems present several physical and chemical disturbances with their own selective forces. Plant species have been used as efficient tools in bio-indication and bio-monitoring studies to indicate water quality and anthropogenic disturbances.

Bryophytes have been efficiently used as bio-monitors of water quality because of their sensibility to the geomorphologic, geologic and ecologic river characteristics, as well as to water pH, alkalinity, conductivity, hardness, oxygen concentration, nutrients content and pollution. Furthermore, aquatic bryophytes are nutritionally independent of their growing substrate, and their nutritional and accumulation patterns are entirely dependent on the water-body characteristics, on ectohydric water transport and on the ecological context.

This work presents a method of bio-indication with aquatic bryophytes that consists of a model relating anthropogenic disturbances and freshwater ecosystems health with bryophyte assemblages and bio-indicator species in the streams and rivers of North-West Portugal.

P2230. Cadmium effects on *Pisum sativum* root nuclei

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Primary seedling roots are an useful experimental system to test the effects of toxic substances, as they quickly respond with morphological alterations and reduced growth, related to the lowering of the mitotic activity at the apex. Moreover, their meristematic cells show a number of aberrations when subjected to genotoxic substances. *Pisum sativum* has a low number (2n=14) of relatively large chromosomes and therefore is a suitable species for genotoxic studies. We administrated cadmium (Cd), as CdCl₂, at different concentrations, to a number of pea genotypes, to test its effect on growth, mitotic activity and induction of mitotic

aberrations. Cv Frisson pea plants were highly susceptible to Cd, as mitotic index of their apices fell to zero after 24 h from Cd treatment. Nevertheless, in 250µM Cd treated seedlings, about 3% of nuclei showed a condensed, prophase-like chromatin, and a 4C DNA content, but did not proceed into mitosis. Prophase-like nuclei have previously been described for animal, fungal and plant cells subjected to stress. Their presence also in plant cells treated with cadmium, may indicate a general occurrence in different stressing situations.

P2231. Analysis of a scrub community on two mining areas in SW Spain

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A scrub community dominated by the species *Genista hirsuta* subsp. *hirsuta* and *Cistus ladanifer* occurs in the Luso Extremadurensis subprovince, included in the Mediterranean-Iberoafrican province, at the southwest of the Iberian Peninsula. This community (*Genista hirsutae*-*Cistetum ladaniferi* Rivas Goday 1956) represents a substitution stage of the oak and cork oaks forests, developed on acid soils.

In our study, this community is found in two mining areas with geochemical anomalies, the only regions with those characteristics in the European territory, the Tinto river mines (Huelva province), and Almaden mines (Ciudad Real province).

We analysed the occurrence of heavy metals (Fe, Cu, Pb, Zn, Hg) into the characteristic plants of the community, and also in the soils. Finally, we compare the floristic composition between the mining areas, and the absorption levels of those metals in the selected species.

P2232. Estimation of Cu, Zn phytoextraction by weeds grown on contaminated sites

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Dept. of Botany, Szeged, Hungary.

Phytoextraction is one of the potential methods for "curing" contaminated places, particularly mine wastes, therefore we have to know whether weeds that grow there could extract or accumulate anorganic pollutants e.g. heavy metals. Four weed species of Asteraceae family (*Tanacetum vulgare*, *Tussilago farfara*, *Ambrosia elatior*, *Erigeron canadensis*) were collected from three sites: uranium spoil, carbon spoil and control. The content of Cu and Zn was measured by AAS and data are given in mg/kg DW. We found that plants generally collected much more Zn than Cu according to higher Zn concentration in the soil. Metal content was usually higher in aboveground parts, almost in leaves, but there were differences between annual and perennial species. Data show that metal concentration depends on the site also e.g. Zn-content was higher on control site and C-spoil in the perennial plants, while in *Tanacetum* and *Tussilago* rather in the spoils.

P2233. Estimation of Cu, Zn phytoextraction by weeds grown on contaminated sites

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P2234. Seed germination as biotests for indication of heavy metals toxicity in drinking water reservoirs

M. Grzesik¹, Z. Romanowska-Duda²;
¹Department of Ornamental Nursery and Seed Science, Research Institute of Pomology and Floriculture, Skierniewice, Poland,
²Department of Plant Growth Regulation, University of Lodz, Lodz, Poland.

The aim of study is to assess the effect of different concentrations of heavy metal salts dissolved in water on seed germination and length of root of several plant species, in order to select the seeds sensitive to pollution and prepare the biotests for indication of heavy metal toxicity in drinking water reservoirs.

Seeds of white mustard, cuckoo, lettuce, cucumber, carrot, parsley, soybean, corn and red clover were germinated on filter paper moistened with solutions of lead nitrate, cadmium nitrate, zinc sulphate or copper sulphate, in concentration of 0,6 - 1280 mg L⁻¹. Every day the germinated seeds were counted and the length of roots and hypocotyls were measured. The obtained results show that the used salts, at concentrations of 0,6-1280 mg L⁻¹, generally did not decrease germination rate of majority species tested. Toxic effect of the used salts has been exhibited by decreased root and hypocotyl length. In seeds germinated during 1 day, white mustard, cuckoo and lettuce, roots and hypocotyls were shorter than in control, on 2nd and 3rd day after seed sowing. Shortened roots and hypocotyls, in the 3rd day, were affected by all the salts used at dosages of 5 mg L⁻¹.

P2235. Seed germination as biotests for indication of heavy metals toxicity in drinking water reservoirs

M. Grzesik¹, Z. Romanowska-Duda²;
¹Research Institute of Pomology and Floriculture, Skierniewice, Poland,
²University of Lodz, Lodz, Poland.

The aim of study was to assess the effect of different concentrations of heavy metal salts on seed germination and seedling growth of some plant species, in order to prepare the biotests for indication of heavy metal toxicity in drinking water reservoirs.

Seeds of white mustard, cuckoo, lettuce, cucumber, carrot, parsley, soybean, corn and red clover were germinated on filter paper moistened with solutions of lead nitrate, cadmium nitrate, zinc sulphate or copper sulphate, in concentration of 0,6 - 1280 mg L⁻¹. Every day the germinated seeds were counted and the length of roots and hypocotyls were measured. The obtained results show that the used salts, at concentrations of 0,6-1280 mg L⁻¹, generally did not decrease germination rate of majority species tested. Toxic effect of the used salts has been exhibited by decreased root and hypocotyl length. In seeds germinated during 1 day, white mustard, cuckoo and lettuce, roots and hypocotyls were shorter than in control, on 2nd and 3rd day after seed sowing. Shortened roots and hypocotyls, in the 3rd day, were affected by all the salts used at dosages of 5 mg L⁻¹.

P2236. Aspect about growth of the vegetation on the anthropic soils from the perimeter of the deposit of domestic waste from Tomesti Iasi, Romania

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Alexandru Ioan Cuza, Iasi, Romania.

The deposit of the domestic waste from Tomesti-Iasi is placed at the river meadow Bahlui on the clayey argillaceous with sandy intercalations from Quaternary period.

The formation of the soil in this region is taking place within in the temperate - continental climate with excessive nuance. These circumstances favour the relative, rapid mineralization of the organic substances with animal and vegetal sources.

The anthropic soil in the perimeter of the deposit has an alkaline pH (9,35-7,47), contains ions H⁺, OH⁻, Ca²⁺ etc and microelements Cd, Pb, Zn etc.

The installation of the vegetation begins with pioneer species from *Solanum*, *Taraxacum*, *Amaranthus*, *Kochia* sort and continues to a final vegetation, represented by spontaneous vegetation associations like: *Kochietum scopariae*, *Atriplicetum tataricae* and *Ivaetum xanthiifoliae*, with a 55 total of cormophyta species. In the storage's slops, algae from *Chlamydomonas*, *Euglena*, *Phacus*, *Oscillatoria*, *Spirulina* and *Chlorella* sort are developing.

P2237. The influence of microcystins on the growth and activity of the katalase, dehydrogenases and phosphatases as a biochemical biomarkers in *Spirodela oligorrhiza*

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¹University of Lodz, Lodz, Poland, ²Department of Ornamental Nursery and Seed Science, Skierniewice, Poland.

The cyanobacterial toxins microcystins are known to affect a number of processes in plant tissues and their presence may have considerable impact on the growth and development of water plants. In this study the *Spirodela oligorrhiza* was exposed for

crude cyanobacterial extracts containing microcystins-LR and -RR. Axenic plants of *Spirodela oligorrhiza* were cultivated for 6 days in a medium supplemented with 0-100 µg L⁻¹ both extracts.

All investigated showed the toxicity with relation to *Spirodela*, changing the activity of investigated enzymes as a catalase, dehydrogenases and phosphatase. The size of changes in the activity of enzymes was relative to the kind of the investigated enzyme and doses of toxins. The growth of plants was inhibited too.

We conclude that the use of enzymes as a biochemical biomarkers is useful to monitor the quality of drinking water reservoirs with of toxic cyanobacterial blooms. *Spirodela oligorrhiza* can be recommended as biosensor for the estimation of water quality.

P2238. Application of chlorophyll fluorescence to detect the PC role in metal tolerance in bryophytes.

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¹Botany Department, Bareilly, India, ²Szent Istvan University, Godollo, Hungary, ³Biological Science, Michigan Technological University, Houghton, MI, United States.

The present work is an attempt to screen to metal tolerance in bryophytes so that same can be recommended for the enrichment of the forest cover as well for the biomonitoring. Fv/Fm values in *Marchantia polymorpha* species ranged 0.67, while same was 0.78 in *Plagiochasma appendiculatum* collected from the same forest cover. Two Chlorophyll fluorescence measurements were taken daily during treatment with lead for the *Marchantia polymorpha* at the interval of four hours, were 0.54 and 0.50 respectively. This photo-inhibition could be due to metal stress. Average Fv/Fm value in *Plagiochasma* species was 0.71 and after four hours same measured 0.75, indicates least effect on photosynthetic function despite metal treatment and therefore, suggests that *Plagiochasma* species is not sensitive to environmental stress as the other species.

High Fv/Fm values in suggest the better tolerance potentials of the *Plagiochasma appendiculatum* and same could be due to better photosynthetic function. On the contrary low Fv/Fm values in *Marchantia polymorpha* indicates impaired photosynthesis could be as a result of metal toxicity.

P2239. Eradication of *Ipomoea carnea* Jacq. (Convolvulaceae), a common and onerous weed, by the application of 2, 4 - D on seedling stage.

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A study was initiated to ascertain the effect of 2, 4 - D application on seed germination and seedlings of *Ipomoea carnea* Jacq. (*Convolvulaceae*), a common and onerous weed, one of the most serious menaces of farmers and agriculturists causing enormous loss of farm output, growing luxuriously both in land and fresh water ecosystems the world over. It is presently well known that the herbicide 2, 4 - D (Dichloro Phenoxy Acetic Acid) arrests the seed germination as well as seedling growth to a great extent effectively. Even a small percentage of the chemical (< 0.1 %) is sufficient to kill the unwanted weed within a few days of application of the chemical. The whole experiment was carried out in our laboratory to suit our conditions and be applied to the field in and around the locality / district. Thus we may be able to arrive at a solution of eradicating this antifarmer onerous weed from the face of the earth for ever.

P2240. Meta-analysis of plant species richness in the vicinity of point polluters: Are pollution effects always detrimental?

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Although effects of air pollution on plants are widely studied, both at the level of community and individual species, the general pattern have not emerged yet, and sources of variation remain almost unexplored. We conducted a meta-analysis of published information on the impact of point polluters on plant species richness and diversity. We were interested in comparison of the effects on different plant groups in different regions, as well as in exploration of factors potentially explaining variation in responses. Primarily studies are highly biased: only one from nearly 100 studies was done in southern hemisphere; 66% of studies were conducted in boreal forests and 23% in temperate forests, while other biomes remain practically unexplored. Most of the studies were restricted to field layer, only 35% involved all vascular plants.

Almost half of the studies were conducted in Russia and published in Russian, which naturally restricts their availability for the international scientific community. Although, as expected, diversity clearly decreased with increase of pollution, the pattern was not uniform among the plant groups. Results will be further discussed.

P2241. Plant and microfungal diversity in an aged hydrocarbon-contaminated industrial soil: from biosensing to bioremediation

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In virtue of their adaptation to local conditions, organisms naturally selected from polluted soils may be suitable candidates for *in situ* bioremediation. Plant and soil saprotrophic microfungal diversity was examined in an industrial dismissed area heavily contaminated with a complex mixture of hydrocarbon pollutants, heterogeneously distributed in the soil. Different plant succession stages were present and were mainly related to the concentration of pollutants in the soil. Pioneer shrub communities and early developed poplar woods (*Populus nigra*) colonised the most polluted areas. Community structure of soil saprotrophic microfungi correlated significantly with vegetation diversity. Association of *Populus nigra* and six microfungal species, isolated from the most polluted spots, significantly reduced pollutant concentrations under (semi)controlled conditions. These results indicate that plant communities can be used for environmental quality assessment in polluted habitats and suggest that rhizoremediation could satisfy the request of biological methods for the restoration of polluted soils.

P2242. Response of native *Pinus pinea* L. to metal pollution in an industrial area

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Needles, wood and bark samples of *P. pinea* L. have been collected along four transects in a heavy industrial area of SW Spain (Huelva) at different distances (0.5, 1, 2, 4, 6, 8 Km) from the industrial complex. Soil samples in the plough layer were taken at each plant sample site. All samples were analysed for Al, Ba, Ca, Cr, Cu, K, Fe, Mg, Mn, Pb and Zn content. Cu, Pb and Zn are accumulated in soils close to the industrial complex. The enrichment factor shows that Cu, K and Ca are the most enriched elements in plants, showing the higher mobility ratio in most of the sampling sites. The next group of elements (Mg, Mn, Pb and Zn) show mobility ratio generally between 1 and 0.1, indicate that their uptake is regulated by internal processes and that pine can be used as a bioindicator of them. Other elements (Al, Ba, Cr and Fe) with mobility ratios mostly < 0.1 are excluded by the plant and are indicative of dust source (Al).

Cu, Fe and Pb content in pine decreases when the distance from the industrial complex increases. Ca content in the different plant parts is bark > needle > wood. Correlation between metal content in *Pinus* needles, bark, wood, total plant and soil is not significant (P>0.05).

P2243. Vegetation variation in two polluted swamps and possible use of plants as bioindicators of pollution

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Degradation of vegetation in two swamps by pollutants from liquor's industries was analyzed by comparison between plants in Boadibo-Douala areas (Cameroon). Floristic inventory was carried out using the base of surveys obtained from relevés from February 1989 to February 1991, and September 1999 to March 2002. The abundance-dominance coefficient permitted to establish for each plant its presence index. Damage index gave the state of each plant. Some physico-chemical parameters of waters in the swamps were also measured. In relation to water quality, significant differences were noted concerning damaged and non damaged plants, BOD₅, total dissolved solid, turbidity and colour. Some sensitive plants in polluted plots exhibited morphological readjustments, chlorosis, necrosis and defoliation. Species like

Commelina sp. And *Mariscus longibracteatus* Vahl seem to be the most resistant to pollution.

P2244. Toxic effects of lead on tip roots onion (*Allium cepa*. L) as biological model to study about contamination for heavy metals.

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Mitotoxic, citotoxic and genotoxic effects induced by lead on onion root cells (*Allium cepa*) were studied at different concentrations and exposure times. Bulbs were cultivated in filtered water changed each 24 h, at $25 \pm 0.5^\circ\text{C}$, in darkness and constant aeration. Treatments were carried out under the same experimental conditions, using watery solutions of lead chloride at 0.25, 0.5, 0.75 and 1.0 ppm to 0, 12, 24, 48 and 72 h. A control was carried out where metal solution was substituted by distilled water. After treatment the meristems were fixed with alcohol - acetic acid (3:1) and they were colored according to Feulgen's technique. An indirect effect was observed between root growth and mitotic index with lead concentration and exposure time. Morphologic alterations, nuclear irregularities and abnormal chromosomal morphology were also observed. That allowed to infer a toxic effect dependent about the concentration and exposure time induced by the lead on the meristems, being able to corroborate the advantages the use of the tips root of *Allium cepa* as biological model to the study of contamination for heavy metals.

P2245. Variability of peroxidase activity and heavy metal content in the needles of Austrian pine (*Pinus nigra* arn.) half-sib lines

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Enzyme activity of peroxidases of total proteins and heavy metal contents were studied in the needles of eight different half sib lines of Austrian pine. The samples were taken from generative seed orchard. Enzyme extraction was performed, by procedure Weckx and Clijsters 1996. The concentration of proteins was determined by Bradford 1976 method. For the measurement of metal concentration needles, the samples were prepared by the procedure for "dry burning". The enzyme activity of peroxidases was monitored in order to determine the effect of genetic variability of half sib lines on one of the key enzymes included in the response to stress, and responsible for the process of lignification in the cell wall. The peroxidases present as soluble in the apoplast or with covalent bond to cell wall, have an important role in lignification, suberisation, healing of injuries, defence against pathogens and atmospheric pollutants.

P2246. Multicriterion assessment of urban geosystems state by methods of bioindication

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The methods of phytoindication include physiognomical, phytooptical, and biogeochemical approaches. They were elaborated and approved by ecology assessment of St.Petersburg geosystems. Physiognomical indication is based on the registration of visible reaction of leaves (chlorosis, necrosis, ect.) on environment contamination, degree of foliation is taken into account also. All these characteristics are included in general index of functional state of green plantation. Albedo of leaves is measured on four channels by contact method. Index of vitality (ratio of visible part to IR-part of spectrum) is calculated. Content of leading chemical elements-pollutants in soil, in leaves and bark of trees reduced to index of summarized contamination (comparison with background data). The summary indexes construction method is the function of normalized initial parameters with assigned weight factors. This method gives a possibility to use amount of characteristics necessary for investigation, take into account importance of their contribution in ecological state of geosystem, and subdivided territory into districts of different ecological stress.

P2247. Flora of the ash hills formed by thermo power plants activity by KEK in Kosovo.

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Introduction: The area of KEK includes a surface of 5000 ha in the central part of Kosovo. From this, 110 ha are covered by ash, produced by thermo power plants activity, which has taken the form of the hills (ash hills).

In older parts of ash hills, a whacking spontaneous flora and vegetation was established. The aim of our researches was to identify all species that vegetate in ash hills, to analyze dominant species, with the aim to be cultivated in the new parts of ash hills.

Material and method: For the study the floristic material is collected in the older parts of ash hills, as well pioneer species in new parts.

Results and conclusions: This is the first study of flora of ash hills in Kosovo. Results shows that during the 2004 vegetative season are recorded: 27 families, 82 genera and 102 species of vascular plants. The richest families with species are: Asteraceae (26), Poaceae (23), Fabaceae (9).

From the list of collected species is concluded that their origin comes from fields and meadows of surrounded areas. Whacking vegetative cover is developed in the older parts of ash hills, which based on exposition and elevation of the hills, acquired the character of associations.

P2248. Use of the Moss *Pinnatella alopecuroides* (Hook.)Feisch. as a Biomonitor of Trace Element Pollution In An Industrial Area Near Mumbai.

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This study is an attempt to use the moss *Pinnatella alopecuroides*(Hook.) Feisch. to monitor the level of trace element pollution in the soil and in the atmosphere. Elemental composition of mosses and soil collected from Mahabaleshwar, a control area near Mumbai was measured. This was compared to samples of mosses and soil collected from a steel factory area near Mumbai and urban area in Karnala Bird Sanctuary. Concentration of eight trace elements As, Cd, Cu, Fe, Mn, Pb, and Zn were measured by inductively coupled plasma spectrophotometry (ICP-OES) and inductively coupled plasma-mass spectroscopy (ICP-MS). The results indicated the following (1)the area around the steel factory is heavily polluted had significantly higher levels of available lead (50 mg/kg^{-1}), Zinc(45 mg/kg^{-1}), and arsenic (1.12 mg/kg^{-1}), than urban and control area. (2)The concentration of trace element in this moss was positively and significantly correlated with the soil availability for lead and zinc, and to a lesser extent for arsenic (loglog relationship). Thus the moss *Pinnatella alopecuroides* could be used as biomonitors for soil pollution of Cd and Zn, and moderately for As.

P2249. Vanadium Pentoxide toxic effect in onion tip roots (*Allium cepa*)

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Vanadium effects on tip roots onion (*Allium cepa*) were investigated in order to value their use as a contamination indicator by heavy metals, analyzing root growth, the Mitotic Index (MI) and of chromosomal aberration (ChA) induction. Meristematic cells were grown in filtered water at 25°C and treatments were done in the same experimental conditions, using pentoxide vanadium solutions at 0.25, 0.5, 0.75 and 1.0 ppm during 0, 12, 24, 48 and 72 h. A control was carried out where metal solution was substituted by distilled water. After each treatment, meristems were fixed with alcohol - acetic acid solution (3:1) and stained using Feulgen's technique. Results showed a negative correlation between concentration and exposure time on roots length and MI. The MANOVA demonstrated a deleterious effect of exposure time and concentrations used. In these were also observed different types of ChA: stickiness, anaphases bridges and c- mitotic effect. Duncan test showed significant increment about aberrations

frequency during the time and concentration. In conclusion vanadium toxic effect on the cell population studied was dependent about concentration and exposure time.

P2250. Remapping of epiphytic lichens in the area of town Ljubljana, Slovenia, as a measure of improvement of air quality in time period 1974-2004

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Air quality was assessed by mapping epiphytic lichens in Ljubljana; Slovenia, first in 1974/75, repeated in 1983/84, and remapped in 2004. During the first mapping the city center was lichen desert. In recent mapping city center is colonised by macrolichens such as *Parmelia sulcata*, *Melanelia glabrata*, *Flavoparmelia caperata*, *Punctelia subrudecta*, *Physcia ascendens/tenella*, *Evernia prunastri*. At sites within the densely settled area the cover of nitrophilous species increased indicating increased air pollution by traffic. At sites around the city some species, present in 1974 disappeared (*Lobaria pulmonaria*, *Sphaerophorus globosus*, *Ramalina fastigiata*, *R. fraxinea*). This indicates that after resolving the problems with dust and sulphur compounds air pollution with nitrogen compounds and organic pollutants remains. Epiphytic lichens responded to changes in level and types of air pollution and they could be used further in biomonitoring of air quality in urban areas.

P2251. Lichens in European Russian cities: myths and reality

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There are many myths around the problem "Lichens in cities". «1. Lichens don't grow in cities». However in 88 cities the European Russia are found more than 600 species (over 50 % species known from this territory). «2. Always there are lichen deserts». But distribution of lichens is of mosaic character in megapolis, big and small cities. This is connected with the presence of suitable habitats and substrata. «3. Lichens differ from higher plants as to their habitat requirements». Like higher plants, lichens are confined to certain habitats (parks, cemeteries, a housing estate etc.). This is proved by statistics. «4. Changes in lichen flora structure are connected with atmospheric pollution». Some other factors are important as well (intensity of human activity, population number per unit of the area etc. «5. Lichens disappear in cities». Besides anthropogenic succession there are natural ones. Approximately 40 % of lichen species persist in one place for a period of 100 years.

P2252. Selected mining dumps in Slanské vrchy mountains - geochemical conditions and their influence on vegetation

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Mining dumps at Slanské vrchy Mnts (Eastern Slovakia) are the specific anthropogenic habitats. According to the representation of ore minerals at dumps and according to their chemical composition in soils, we supposed higher concentration of some elements (As, Cu, Hg, Pb, Sb).

The results confirm the migration of heavy metals and also their accumulation in environment. Our aim was to catch also natural succession of plants at dumps, their development and stand conditions at individual localities. We discovered *Agrostis capillaries*, *Acetosella vulgaris*, *Amelanchier lamarkii*, *Betula pendula*, *Fagus sylvatica*, *Salix caprea*, *Silene vulgaris* and *Tussilago farfara*. We confirm some differences between the growth of young and old dumps. In plants the content of mercury and arsenic exceed the values of ash. The most frequently it is exhibited in the case of *Agrostis capillaris*. In another species there is a high concentration of Hg from each locality.

P2253. Monitoring environmental pollution by plants in the spill-affected Guadiamar valley (S. Spain)

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Higher plants have been widely used for monitoring the air and soil pollution. In April 1998, the Guadiamar valley (S. Spain) was severely affected by a mine spill. We have analysed the concentration of As, Cd, Cu, Pb, Tl and Zn in different organs of

several plants. Leaves of the white poplar (*Populus alba* L.) accumulated Cd (up to 14 mg kg⁻¹) and Zn (up to 1200 mg kg⁻¹), and were used as pollution biomonitors. Leaves of wild olive (*Olea europaea* L.) and holm oak (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp.) were also used as pollution biomonitors. Some herbaceous species, such as *Cynodon dactylon* (L.) Pers. *Hirschfeldia incana* (L.) Lagrèze-Fossat, *Raphanus raphanistrum* L. and *Plantago lanceolata* L., also accumulated some trace elements. Other organs, besides leaves, can be very relevant for ecosystemic monitoring. For example, the fruits of olives and oaks are consumed by many animals in the Mediterranean forests. In another example, it was remarkable the high accumulation of Tl found in the floral structures of the Brassicaceae herb *Hirschfeldia incana* (up to ca. 50 mg kg⁻¹) while the leaves of the same plants accumulated much less (only 2 mg kg⁻¹).

P2254. Testing water-mosses for their indicative value at three Austrian rivers (Traisen, Große Tulln and Perschling)

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In the course of this study it was evaluated whether the presence of water-mosses correlated to water quality (in terms of eutrophication), chemical composition, velocity, water-temperature, the structure of the waterbed and the banks, anthropogenic modification of the water-body and the presence of other organisms (algae). It was aimed to test the use of water-mosses as bio-indicators for these parameters.

The study was conducted at three rivers of the foothills of the Austrian Alps (Traisen, Perschling and Große Tulln) showing differing water regimes.

The occurrence of water-mosses was recorded at transects across the river, at sampling points every 2,5 km and additionally, at measurement points of the local government. Recording followed a plotless sampling design, with a distance of 10 cm for each sampling plot, additionally water-mosses at the near surroundings were also listed.

Correlation between each investigated parameter and the mosses found were calculated by a range of statistical procedures (e.g. multiple regression analysis). The application of water-mosses as bio-indicators is discussed.

P2255. Biomonitoring of road traffic emissions by mosses

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Mosses were used as biomonitors for estimating heavy metals and PAHs deposition deriving from road traffic emissions in Austria. A road tunnel experiment was performed and a series of transects along major roads were investigated. Twenty-two elements, mainly heavy metals, and the sixteen EPA-PAHs and coronene were analysed. Highest average enrichment factors were obtained for most PAHs, especially benzo(g,h,i)perylene, coronene and benzo(a)anthracene, and among the other elements for Sb, Pd, Mo, Pt, Cr, As and Cu. There was a significant negative correlation to the distance from the road for most of these elements. The rate of decrease followed a reciprocal curve. Traffic density, distance from and elevation of the road were the most influencing factors. There were nearly no significant elevated deposition at road distances more than 200 m. Nevertheless, at heavily frequented roads, raised deposition of some elements were found even at a distance of 600 m. On the basis these results a regular road traffic monitoring system was established along 46 roads in Austria.

P2256. Phytoindication of the Surface Waters of the Canyon of the River Smotrych (NNP "Podilsky Tovtry")

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The research was held on the territory of National Nature Park (NNP) "Podilsky Tovtry", the biggest in Ukraine and the second big as for the territory in Europe.

In course of the research the spreading and ecological peculiarities of the growth of macrophytes-indicators according to hydrological and hydrochemical conditions were established. The index of phytoindication (If) was indicated, which characterizes the condition

of waters of the canyon of the river Smotrych as unsatisfactory. The valuations of condition of water ecosystem under of influence of anthropogenic press was given in the comparison of data of phytoindication researches with hydrophysical and hydrochemical parameters.

On the whole phytoindication researches are rather actual especially on the nature protected territories. They give the opportunity to define integral level of pollution as well as general state of water ecosystems. At the same time we have an opportunity to make the discreet control with the help of hydrochemical methods and only in the definite periods of time. And some changes between observations

P2257. *Pistia stratiotes* L. (Araceae) morphological changes in non polluted and polluted ecosystems in Douala areas (Cameroun)

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Douala aquatic ecosystems are submitted to pollutants that affect distribution and morphology of plants such as *Pistia stratiotes* L. (Araceae). 40 families, 100 genera and 116 species have been recorded in Twenty swamps. Species recovery where done by abundance-dominance indexes in 0,3 ha. Physico-chemical values in swamps are variable : pH (6.32 ± 0.28 to 7.24 ± 0.10), Conductivity (129.98 ± 26.24 to 3334.70 ± 7.35 meq^l⁻¹), TDS (1.29 ± 0.26 to 33.34 ± 0.07 meq^l⁻¹), NH₄⁺ (0.15 ± 0.07 to 18.32 ± 5.18 mg^l⁻¹), PO₄³⁻ (9.36 ± 2.64 to 42.67 ± 12.07 mg^l⁻¹), NO₃⁻ (8.78 ± 2.48 to 27.61 ± 7.80 mg^l⁻¹), Cadmium (0.001 ± 0.00 to 0.37 ± 0.01 mg^l⁻¹), Oxygen demand (0.27 ± 0.07 to 0.73 ± 0.19 mg^l⁻¹), BOD₅ ($38,0 \pm 3.5$ to 212 ± 10.6 mg^l⁻¹) The higher value and the lower value of each parameter were significantly different. Biological parameters are also variable: leaves mean number (7.61 ± 1.93 to 13.53 ± 3.05), root length (5.15 ± 1.43 to 20.53 ± 14.62 cm), leaf area (7.06 ± 2.23 to 86.89 ± 12.23 cm²). *P. Stratiotes*, less abundant in sites receiving organic matters, hydrocarbons and Cadmium with flowers and seeds absent or scarce can be used for monitoring in polluted areas and help to purify water.

P2258. Algae in oil-cleaning carts: Who and how survive in extremely polluted waters?

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Effects of oil products and 18 other chemicals on phytoplankton biodiversity and biomasses in three carts of water cleaning system of Kirishi State Oil Company, Leningrad district were studied in 2003-2004. Total number of algae species were more than 70 taxons with only 9 species belonging to Cyanophyta (*Oscillatoria granulata*, *O. agardii*, *O. ingraca*, *O. tenue*), Chlorophyta (*Coelastrum microporum*, *Chlorella vulgaris*), Cryptophyta (*Cryptomonas rostrata*) and Euglenophyta (*Euglena anabaena*, *Euglena bucharica*) as the most common forms. Biomasses of dominative plankton cyanobacteria negatively correlated with SO₂ (R=-0,636; p=0,026), dried rest (R=-0,588; p=0,044), Fe (R=-0,351; p=0,039), total phosphorus (R=-0,355; p=0,037) and Mn (R=-0,609; p=0,035) and positively correlated with biological consumption of oxygen (R=0,649; p=0,00004) in the carts. Synergic effects of oil products pollution on algal community in the carts are also discussed.

P2259. Morphological and physiological parameters of *Carex hirta* under oil pollution of soil.

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Morphological and physiological reactions of *Carex hirta* under soil pollution by oil were investigated. *Carex hirta* - long-root species, most stable to the hard conditions of ecotops, polluted by oil. Plants were harvested in 15 l pots. Oil was brought to the soil in the following concentration - 50 ml/kg (medium pollution) and 100 ml/kg (strong pollution). Control - soil without oil. After two month of growing plants we analyzed morphological and physiological parameters: growing and biomass accumulator, complex of pigments, POL activity. We investigated, that strong oil pollution due to growth and development brakes, decreasing of fresh and

dry weight of plants, *a* and *b* chlorophyll content, increasing POL activity and carotenoid concentration. All this suggested about physiological adaptation of *Carex hirta* to oil pollution.

Investigation of physiological and biochemical ways of stable plants adaptation to oil pollution is important to scientific recommendation for phytoremediation of territories polluted by oil.

P2260. Relationship between metal concentration in moss tissues and deposition fluxes: example of a french remote area.

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The use of mosses to estimate atmospheric trace element deposition is studied. Annual deposition rate (sum of wet and dry bulk deposition) is compared to the rate estimated by mosses (*Scleropodium purum* (Hedw.) Limpr.) collected in the same area. This estimation is obtained by the product of the concentration in the one year growth segment (upper 2 cm of the plant) with the annual biomass production per area of the plant.

The ratio value of moss to bulk deposition varies with the elements and gives the following order: Mn > Zn > V > Pb > Fe > Al, where Mn deposition is overestimated whereas Al deposition is underestimated by mosses.

The main processes observed which could explain the differences between real and estimated deposition are listed below.

- A comparison of the metal concentration in the apical and basal parts of the plant shows a specific bioconcentration of Mn and Zn in the youngest tissues.

- The physico-chemical state of the element (dissolved, particulate) seems to influence its capture and retention by the moss.

- Following an artificial contamination of the moss carpet, the retention time of the elements sprayed is lower than one year.

P2261. A test system with radish (*Raphanus sativus* L.) to study biological effects of low-frequency electric and magnetic fields

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The applicability of plant-based test methods to be used as bioindication systems for elevated electric and magnetic field strengths was studied for two years. Additionally the relations between specific ecophysiological reaction patterns and electromagnetic fields were analyzed. The most versatile test procedure consisted of a standardized cultivation of young radish plants for two weeks under the field conditions to be tested. In a next step, leaf chlorophyll fluorescence had to be measured and the results had to be fed as inputs into newly developed neural network models. The model outputs provided reliable evidence for the existence, type and strength of the electromagnetic field (0-300 Hz) if the detection limit of 3 μT or 1 kV.m⁻¹ was exceeded. This test system was optimized for the use as indicator system under real-world conditions. The reactions of radish were mostly non-linearly affected by field strength. At low and medium field strengths growth and photosynthetic parameters frequently were promoted; at higher field strengths (>30 μT or >6 kV.m⁻¹), however, reductions prevailed.

P2262. Oxidative stress induced by trivalent and hexavalent chromium species in *Fontinalis antipyretica* Hedw.

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Due to its widespread use, chromium, has become one of the most abundant pollutant in aquatic and terrestrial ecosystems. It can be found in several oxidation states even if the most common and stable forms are the trivalent Cr(III) and hexavalent Cr(VI) species. In plants, Cr(VI) and Cr(III) are both able to cross biological membranes. Then, inside the cells, they are both involved in reactive oxygen species (ROS) production. Apart from generating reactive oxygen species (ROS), Cr(III) can also inhibit metallo-enzymes systems.

We wondered whether Cr(VI) and Cr(III) could induced an oxidative stress in the aquatic moss *Fontinalis antipyretica* Hedw. Chromium was added to exposure media as chloride (CrCl₃·6H₂O), nitrate (Cr(NO₃)₃·9H₂O) or potassium dichromate (K₂Cr₂O₇). After 24 hours, 100 mg fw moss samples were analysed for superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase activities, GSH/GSSG ratio and chlorophyll

contents. Chromium was localized in cells through TEM-XF analysis.

All variables responded to Cr(III) and Cr(VI) exposures, suggesting an ROS-mediated metal toxicity.

P2263. Subcellular comparison of the toxic effects and distribution of cadmium and lead in plants

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The toxicity of heavy metals was estimated by observations in epidermal cells of *Allium cepa* onion bulb scales. Observations of cytoplasm stream flows in cells allowed arranging the investigated metal ions by order of their toxicity in the sequence: Pb<Zn<Cd. A large difference in the toxicity of cadmium and lead was demonstrated. The magnitude of this difference was 4-fold in relation to cytoplasm flow, and 63-fold, in relation to the total contents of cadmium and lead in cells. After fractionation of tissues and enzymatic digestion of cell walls it was confirmed that the largest amount of cadmium was in cell walls, in contrast to lead, of which 97.8% was in intracellular compartments other than the cell wall. Explanation of these results was possible by locating lead in cells using electron microscopy. This study showed that an effective detoxifying mechanism exists in the epidermal cells and that it can be one of the mechanisms explaining the lower toxicity of lead than cadmium.

P2264. Adaptation of *Biscutella laevigata* L., a metal hyperaccumulator, to growth on a zinc-lead waste heap in southern Poland

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Biscutella laevigata is an herbal member of the *Brassicaceae* family and a typical mountain species. It has recently been identified as a hyperaccumulator of Pb, Cd and Tl. Its northern reach runs through Poland, where it is found only in the west Tatra Mountains and on calamine waste heaps in the vicinity of Olkusz. The peculiar distribution of this species in Poland prompted us to undertake studies to identify the traits that allow this typically mountain species to grow so robustly on industrial waste heaps near zinc and lead smelters in the vicinity of Olkusz. The both of plant populations were compared. It was found that the mountain and waste heap populations of *B. laevigata* differed significantly: the waste-heap population is more tolerant to heavy metals (Pb, Zn, Cd). In the presence of both Zn and Pb, growth of the waste-heap plants was stimulated, while under the same conditions, growth of the mountain population was inhibited lower than 50%. Histochemical detection of metals- dithizone in tissues showed that there were taken up by the root hairs and transported through vascular bundles to the leaves (the epiderm and hairs). It may be a mechanism of metals detoxifying.

P2265. Occurrence of Central European threatened plants in water man-made habitats in Slovenia

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The data of Central European threatened vascular plants (listed on Annexes II (=Natura 2000 species) and IV of the Habitats Directive, and Appendix I of the Bern Convention) have been analysed in FloVegSi database (Flora and vegetation of Slovenia) regarding their occurrence in man-made water habitats. The following 6 species occur in Slovenia: *Eleocharis carniolica* Koch, *Lindernia procumbens* (Krocker) Philcox, *Marsilea quadrifolia* L., *Salvinia natans* (L.) All., *Trapa natans* L., *Typha schuttelworthii* Koch & Sonder. Sites and vegetation community conditions were compared with the records of their natural occurrence. 5 threatened species are present in both habitats: natural and man-made. In the last century *Marsilea quadrifolia* only in man-made water bodies (fish-ponds) has been recorded. Man-made habitats are important for keeping the gene pool of many threatened species but should not become the only solution for their survival.

P2266. "Ecoflora of the Ukraine" - basic indication of ecological factors

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Plants reacting on the changes in the environment have long been used as indicators of the environment. A number of phytoecological scales have been elaborated. Standardization of ecological scales (Ellenberg, Ramenski, Tsyganov, Landolt, Zarzycki et al.) (Didukh, Plyuta, 1994) enabled to make generalizations about ecological conditions of growth of species. This information served as a base of the multivolume edition of "Ecoflora of Ukraine" (3 volumes published so far). Descriptions of biomorphological traits; type area; phytocoenotic peculiarities; ecology; consortional links, influence anthropogenic factors; economic, landscape, and indicator importance; data on species protection and renewal; drawings and maps of distribution in Ukraine are given in the edition. Obtained ecological characteristics are used for evaluation of indicators of state, changes, and interrelations of ecological factors on the base of elaborated synphytoindication method. The method enables to evaluate species econiches, prognose vegetation changes, create ecological maps and elaborate corresponding prognostic models.

P2267. Correlation of angiosperm diversity with diversity of selected plant families in Burkina Faso

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Digitization of specimen label data for Burkina Faso from the Herbarium Senckenbergianum (FR) and the Ouagadougou University Herbarium (OUA) led to a database including 18,000 specimens. Field observations from about 3,700 relevées (Braun-Blanquet method, BIOTA standard relevées, simple inventories) were added to the database and together used to model potential species distributions for Burkina Faso on a 10 minute grid. GARP was used with temperature, precipitation, humidity and elevation data. Based on these modelled distributions, overall angiosperm diversity is correlated to the diversities of the 10 families with on average the highest species numbers per grid cell (Poaceae, Fabaceae, Cyperaceae, Rubiaceae, Combretaceae, Euphorbiaceae, Mimosaceae, Asteraceae, Caesalpinaceae, Malvaceae). The value of these families as indicators for assessing biodiversity is discussed. The results are compared to similar analyses based on relevées only.

P2268. Characterization of macrophytes in man made canals in Hungary

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In the frame of the "Multifunctional Integrated Study Danube: Corridor and Catchment" the macrophyte vegetation was characterised in five man-made canals (Danube-Tisza Canal, Main Canal, Apajai-canal, Sós-ér) in the Danube-Tisza lowland in 2002. The survey technique followed the Kohler-method, which is in accordance with the respective European Standard EN 14184, and the regulations of the Water Framework Directive. One aspect of the study was to prove that the method, which is in use in all Danube countries today, is also suitable for characterising the aquatic vegetation of anthropogenic canals.

The standardised field survey, and data processing, methodology guarantees that results are comparable between various locations. Findings include information on species number in canals as compared to other water body types in the Hungarian reach of the Danube and on distribution types of dominating canal species, of species of more conservation interest, and of neophytes.

P2269. Change of areas of rare and vanishing species of plants on territory Volynsk Polesye (Northwest Ukraine)

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The flora of Volynsk Polesye includes 311 rare and vanishing species (nearly 30 % of the flora) consisting of 136 European, 63 Holarctic, 56 Boreal, 27 Subpontic, 11 Submediterranean, 8 Subatlantic, 7 Cosmopolitan, 3 endemic species, as well as 4 tertiary, 26 glacial, 32 interglacial and holocene's relics. Comparing the data on distribution of these species in XIX century on territory Volynsk Polesye with the modern data, we studied their change of

areas during last 150 years. Among them 12 species (*Cardamine hirsuta*, *Empetrum nigrum*, *Leymus arenarius*, *Linnaea borealis*, *Ranunculus nemorosus*, *Rubus chamaemorus*, *Orchis ustulata*, *Pedicularis exaltata*, *Marsilea quadrifolia*, etc.) already have vanished, 36 species are endangered, 107 species, mainly boreal, reduce the areas, 170 species have stable areas, 27 species expand the areas on territory Volynsk Polesye from southern and western regions. These changes are connected to anthropogenous transformation (drainage of bogs, cutting down of woods) and it is possible with global warming of a climate.

P2270. Analysis of the species distribution in peatbogs from NW Iberian Peninsula using two statistical approaches

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The effects of environmental parameters on the distribution of peatbog vegetation were assessed using different approaches. The peatbogs studied are situated at the southern limit of the optimum peatbog plant communities, in Xistral and Ancares Mountains (NW Iberian Peninsula). These small peat deposits are scattered throughout this area, where some of the peatbog characteristic species remain as relict populations and also many endemic species are present.

To characterise the vegetation, linear transects were established on each bog and monitored monthly during the spring and summer of 1999-2003. Floristic inventories were carried out in each square metre along these transects and environmental parameters were measured as well. The resulting data were examined using multivariate PCA and CCA analyses, and the syntaxonomical method of the ecological profiles. Finally, our results were compared with published data, and their utility for establishing management plans to assure their conservation was evaluated.

P2271. Ecological and floristic analysis of the Pteridoflora of the Valley of Mexico

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An analysis of 113 species of Pteridophytes in the vegetation types from the Valley of Mexico is carried out. We found that 54 % of the species occur in the cloud forest, 45 % in conifers forests, 33.6 % in *Quercus* forest, 34.5 % in grasslands, 28 % in xerophilous scrubs, 22 % in pine-oak forest, 3.5 % in *Quercus* scrub, and 1.8 % in aquatic as in halophilous vegetation. 35 species are restricted to a single community, most of them to cloud forest (13 species). The affinities between the Pteridofloras of the different plant communities were analyzed through a cluster analysis (UPGMA). The temperate forests formed a cluster and on other hand the semiarid vegetation; halophilous vegetation was scarcely similar to other communities and the aquatic vegetation had no relationships with anyone. It was interesting that the pine-oak forest did not form a group with pine forest nor even with oak forest, because apparently it posses an own Pteridoflora, this fact supports the idea that it is an independent vegetation and it is not just an ecological or successional transition between the pine and the oak forests. Only five genera represent the 47.8 % of the species.

P2272. The evaluation of the flora and vegetation in the flood plain of Lower Tisza on the basis of ecological indicator values

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The study site situated in the flood plain of Lower Tisza (Hungary) was approx. 45 km². The distributional ratio of the main habitat types is as follows: permanent and temporary lakes and wet meadows 3,8%, willow shrubs 0,07%, gallery forests 26,1%, degraded gallery forests 19,6%, pastures with trees 7,1%, softwood plantation forests 19,0%, agricultural areas 9,6%, cleared forests 1,3% and others 13,5%. The predominating plant species are disturbance tolerants followed by competitors and generalists. These plants require submontane broad-leaved forest climate, wet and neutral/alkaline soil without salt and a lot of sunlight. The distribution area of most of the species is eastern Central Europe and they occur in soils rich in nutrients. Out of the six coverage categories of the invasive species the category 20-50% occupies about of the half of the area. The average Németh-Seregélyes nature conservation value counted for the study area - weighted

with the total area of categories - is 2.24 (the worst value is 1, the best is 5).

P2273. Yew (*Taxus baccata* L.) stands as priority habitat for biodiversity conservation in Mediterranean ecosystems: a case study from Northern Sardinia (Italy).

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Yew (*Taxus baccata* L.) is a long-lived circumboreal tree species with a limited range throughout the Mediterranean basin, where it is restricted to mountain areas in small populations. Following the EU Directive 43/92 "Habitat", Sardinian yew stands can be ascribed to the priority habitat 9580. In northern Sardinia, yew is distributed in 6 mountain areas located in bioclimatic islands, with 11 main populations of different size. Adult density (from 5 to 460 ind/ha) is negatively affected by altitude (from 300 to 1300 m asl). Seedling and sapling densities (respectively from 0 to 8700 and from 0 to 1174 ind/ha) are both positively correlated to adult density and average age (expressed as DBH and ranging from 79.1 to 780 mm). Mortality (from 0 to 41.8 dead ind/ha) is also positively influenced by adult density and average age. Recruitment is conditioned by the presence of flesh fruited shrubs and trees. Because of the presence of mountain endemics and boreal-temperate relict species in yew stands, they show higher a-diversity in comparison to *Quercus ilex* L. and *Q. pubescens* L. ones and confirm their role as biodiversity hotspots in Mediterranean mountain ecosystems.

P2274. About "hot-spots" analysis for the rare plants conservation within the Ukrainian Carpathians

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At present time the Mountain Flora of the Ukrainian Carpathians includes more than 150 rare or endangered Vascular Plant species, and within them about 50 species are concentrated in 10 to 15 "hot-spots" as the sectors of the greatest diversity of the most endangered taxa (Ziman et al., 1994-2004). Within them we regard the summits of Bliznitsa Mt. (Svidovets Range), Petros (Chernogora), Pip Ivan Marmaroshski Mt. (Marmarosh) and Negrovets Mt. (Gorgany) as the highest essential high-mountain "hot-spots" because of growing there 10 to 15 rare species within the same community (viz., unique *Anthemis carpatica*, *Astragalus krajinae*, *Gentiana nivalis*, *Campanula subcapitata*, *Salix alpina*, *Thalictrum transsilvanicum* and others). Besides, the Geredjivka Mt. (Gorgany) is the very valuable middle-mountain "hot-spot" because *Gentiana verna* and *Carex davalliana* grow within the Ukrainian Carpathians only here, and *Primula farinosa* disappeared from this relict community about 30 years ago (Ziman, 1964, Ziman et al., 2001).

P2275. A comparative study of lichen diversity in lines and wooden-meadows, Norrtälje, Sweden.

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An inventory of lichens in lines and wooden-meadows were performed in the rural district Norrtälje north of Stockholm to compare the lichen diversity in the two habitats. The inventory were performed in two lines and four wooden-meadows. The lines had a higher species diversity of lichens than the wooden meadows. The habitat was the only character that were significant different between the six different localities. Other characters that were measured were the crown-width of the trees, circumference of the trunk, distance to dust, and distance to the closest tree of the same species. The lines are located by gravel-roads and have therefore a richer environment than the wooden-meadows. The dust that are produced by the gravel-road make the environment on the tree trunks richer in nutrients for the lichens.

The wooden-meadows are a habitat typical for the area around the Baltic sea. They are meadows with some broad-leaved trees on them that are (or at least were) cut in a specific way. Every third to fifth year the tree crown were cut down and the branches were given to the animals as food, this is in Sweden called "hamling".

P2276. Effect of the Cañaveral-Arrecifes path (Tayrona National Natural Park) over the surrounding vegetation

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In order to establish what would be the effect of the Cañaveral Arrecifes Path over the surrounding vascular vegetation (Tayrona National Park, Colombia), we analyzed the changes in structure, diversity and plant composition in a spatial gradient from the edge of the path to the inner part of the forest. In 10 different areas we established 7 transects (50 x 2 meters) at 0, 40, 80, 120, 160, 200 and 500 m of distance. A small variation was obtained for height and dbh curves between distances, indicating an invert "J" trend, which suggest that the forest is still well preserved, although there exists absence of some individuals of intermediate classes because of previous tree cut down. We found in total 227 species, but the values of the predominance indexes showed that *Anacardium excelsum* and *Ficus pallida* are dominant. The diversity varies between the edge and the inner part of the forest in a significant way (KW:18.11; $p < 0.01$), notwithstanding it is not high as one would expect for a tropical rain forest. The richness, dominance and abundance, also were highly significant ($p < 0.01$) between distances. These results suggest that the path is an important fragmentation source.

P2277. Assessment of woodlands for crop wild relatives conservation

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In our study we assessed the woodlands of the Leningrad region from the point of view of the *Rosaceae* Juss. family crop wild relatives (CWRs) *in situ* conservation.

For evaluating the share and importance of CWRs in the vegetation cover, we derived the phytocenotic significance index for each species on the basis of its frequency and abundance values. The conclusion about the conservation significance of an individual habitat was made on the basis of the biocenotic success of CWR species, conservation priority of these species, and the importance of the woodlands.

Assessment of CWR resources was carried out both within and outside the protected areas of the region. The high concentration of different valuable CWR species within the protected areas indicates to the necessity of additional protective measures regarding these areas. The woodlands, rich in rare CWR species, and/or possessing unique or diverse populations of these species outside the protected areas, were recommended for establishing *in situ* genetic reserves.

P2278. Influence of human damage actions and soil characteristics in the establishment and development of secondary forest in sand coastal plain vegetation (Restinga Forest) of the littoral area in the state of São Paulo, Brazil

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The sand coastal plain vegetation (Restinga Forest) has been described as an ecosystem associated with the Atlantic Forest, constituted of mosaics, which occur in areas of great ecological diversity. This work was carried out in the Anchieta island, a State Park in the city of Ubatuba in the northern coast of the state of São Paulo, Brazil (45°02' - 45°05' W and 23°31' - 23°34' S). The soil humidity associated with the type and intensity of human damage actions, as well as the natural time regeneration, creating the soil fertility patterns, determined the vegetation physiognomies found in the mosaic of that secondary forest. Based on such information and the plasticity of the most representative plant species, was suggested a model of plantation for natural recovery of gallery forest in Restinga ecosystems.

P2279. Relationship between threatened species richness, habitat diversity and landscape structure: a case-study from a National Park in Northern Portugal

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The lack of information on biodiversity patterns and on ecological processes underlying those patterns is often an obstacle in management plans for natural and semi-natural landscapes. This is particularly critical in protected areas, which are repeatedly used as models for the development of methodologies regarding the protection and management of biological diversity, since the required ecological data is most often available for these areas and the moderate human impact allows different levels of disturbance to co-exist.

This work addresses the influence of landscape structure and distribution patterns of natural habitats on the local richness of threatened bryophyte and vascular flora. Both GIS-based spatial analyses and statistical techniques are used, since they have been proved to be powerful tools on the establishment of relationships between the variation of ecological factors and biodiversity patterns. A synthetic model relating species, habitat and landscape patterns is proposed using data from Peneda-Gerês National Park (Northern Portugal). The significance of this model for management purposes is discussed.

P2280. Old growth stands in riverine forests of the Transcarpathian lowland (Ukraine)

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In spite of the dramatic reduction of lowland riverine forest in the Transcarpathian lowland (NE Hungarian Plain, Ukraine) highly valuable old growth stands of riverine hardwood forests have survived.

The sites are characterized by alluvial gley soils and high ground water table or flooding connected with snow melt in spring or rain periods all over the year.

The analysis of a permanent plot of 0.2 ha shows an uneven aged stand with an upper canopy dominated by *Quercus robur* and *Fraxinus angustifolia* subsp. *danubialis* with dimensions of more than 40 m height and 130 cm breast height diameter/BHD). The lower canopy is represented by a high number of stems of *Acer campestre* and *Carpinus betulus* up to 40 cm BHD, *Ulmus minor* and *U. laevis* are less frequent. The stem diameter distribution is typical for unmanaged woodland.

Beside the monitoring of the forest renewal processes the occurrence of rare or Red List species e.g. *Epipactis albensis*, *Hottonia palustris*, *Thelypteris palustris*, *Urtica kioviensis* are further reasons for the urgent conservation need of this largely overlooked biodiversity hot-spot and largest surviving remnant of Central European Riverine forests.

P2281. Characterization of management unities (biótopos) of a conservation unity based on landscape ecology.

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The Santana Hill, concerning its natural resources, represents an important area covered by woods, fields and native brushwood located in south of Brazil. It comprehends an area of approximately 1000 hectares, from which around 360 ha constitutes the Conservation Unity. Because it is a natural area amid urban centers, the types of potential uses of this Conservation Unity extend themselves to natural environment conservation use, where certain zones could be established where such uses as leisure, environment education, among others, should be allowed. The recognition of possible zones with potential diverse uses, which will constitute the management unities ("biótopos") of the area, will be achieved utilizing the physical characteristics (declivity, altitude, solar exposition and humidity of soil), spatial structure of the landscape (landscape rates), and, we will relate the variants from the ecological processes (characterizing the dominant vegetal species) of the landscape elements. The relationship between the valuation of association vegetables and the valuation of spatial structure of the landscape will show the management unities.

P2282. How Taxonomy contributed to the Conservation of Natural Ecosystems in Suriname

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The Republic of Suriname, has about 90% of its land surface (164,600 sq.km) covered with forest. Since the Nature Conservation Law was promulgated in 1954, 11 Nature Reserves, 1 Nature Park, and 4 Multiple-use Management Areas have been established. They cover 13 % of the land surface. In 1998 three existing Nature Reserves (Tafelberg, Eilerts de Haan Gebergte and Raleighvallen / Voltzberg) have been linked into the Central Suriname Nature Reserve, (CSNR), with an area 59,053 square km. In 2000, the CSNR was put on the UNESCO World Heritage List. During the Guayana Shield Priority-setting Workshop in 2002, organized by the Guiana Shield Initiative, (GSI), the Netherlands Committee for the World Conservation Union (IUCN), and Conservation International Washington (CI), the CSNR has been designated as a Priority 3, on a scale from 1-4 (high for Biological Priorities and Pressure). The focus will be on the endemic and rare plant species in the CSNR, and their importance for the fauna.

P2283. Monitoring the effects of human trampling disturbance on *Senecio incanus* L. and *Salix herbacea* L. fragmented small populations in the N-Apennines (Italy).

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In the highest elevations of the N-Apennines, some species growing in small fragmented populations are considered locally rare for phytogeographical and ecological reasons. These populations are at or toward the southern border of their range, are largely isolated from populations growing in the Alps, and are dependant upon late season snow cover. An overall trend of increasing visitation and associated natural resource impacts have been detected in the N-Apennines, in particular in the Mount Prado area of Tuscan-Emilian Apennines National Park. In the Mount Prado area, *Salix herbacea* L. and *Senecio incanus* L. subsp. *incanus* are sensitive to environmental stresses and are in great danger due to human trampling disturbance and to the global warming phenomenon. Since 1999, high visitor impact stands and unimpacted stands were monitored by our research team in permanent quadrats. The measured variables included: total vegetation cover; cover and fruit number of *Senecio incanus* and *Salix herbacea*; late season snow cover.

P2284. Macrophytes and flood impact in a Danube anabranch, a "Natura 2000"-protected area in Linz (Austria)

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In 2001 and 2002 the aquatic vegetation was surveyed in the "Mitterwasser" anabranch of the River Danube near Linz (Province of Upper Austria). Comparative field studies using the Kohler methodology were carried out four times per vegetation period in order to show fluctuations in the relative plant mass, the species composition, and distribution of the occurring macrophytes. An additional survey in 2003 showed changes in the vegetation following the flood in August 2002.

The present information is of special importance as a reference to possible future man-induced changes in this system. Today this part of the former Danube floodplain is separated from the main river channel by the levees of the run-of-river power station Abwinden-Asten near Linz. An artificial channel system is planned to ameliorate surface and ground water conditions, but the permanent inflow of cold river water may delay the germination of important aquatic plant species.

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P2285. Biodiversity value of Mediterranean temporary ponds in North-western Sardinia

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Temporary ponds are small and shallow water bodies, which exist only in a part of the year. Despite their vulnerability, their presence is often favoured by traditional pastoral and agricultural activities. The flora is mainly composed of amphibious Mediterranean species including numerous endemic and rare. The case study illustrates the botanical biodiversity of temporary ponds in a protected area (Asinara National Park) and in a close unprotected one where traditional livestock activities are still practised. Different pond types, belts (inside the ponds) and communities were

analyzed. Alpha (species richness and evenness) beta and gamma diversity were assessed for each of them in terms of wetland macrophytes. In addition, rare species or unique to an assemblage were listed. Comparison inside and between the areas and with the entire regional gamma diversity were made in order to point out the conservation value of each assemblage and each area. Threats and possible mitigation were also identified. The results allow to define strategic conservation goals for temporary ponds across the landscape as a whole.

P2286. Field guide to the plants of the páramo of Cajas (Ecuador): an example to encourage and support biodiversity conservation

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The field guide to the plants of the páramo vegetation of Cajas National Park is the first illustrated guide to any protected area in continental Ecuador. It was produced in collaboration with local biologists and with support of park administrators. The guide, in Spanish and English, comprises one hundred species, is written in simple but accurate language and illustrated with color photographs. It is intended as an identification tool for the non-botanist. It will enable visitors to the Park, local guides, and authorities to recognize, name, and understand the use of the wild plants and gain an increased appreciation of the flora of this threatened ecosystem and of Ecuador in general, motivating them to better manage and conserve both the plants and their environment. We believe that through education we can conserve species in Ecuador. The guide and additional materials are also available online and reviewers and users have found the website to be one of great quality and merit.

P2287. Differentiation of woodland communities in relation to wood age in the Ojców National Park (Southern Poland)

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Ojców National Park (2145 ha) was established in 1956, in aim of protection floristic and community richness of the Krakowsko-Czestochowska Upland. This terrain is specific for rich relief and lime rocks. It causes that occur here kserothermic grasslands, scrubs and woodlands communities with large number species of plants. Relationship between types of forest's communities and age of woodland were studying by comparisons map of plant communities and map of woodland ages. First one was done in support of Braun-Blanquet method. Map of woodland age was prepared independently by studying old and actual cartographic, historic and botanic documents. It was found that ancient forests consist of well-developed forest communities: Dentario-glandulosae-Fagetum, Phyllido-Aceretum, Pino-Quercetum and Tilio-Carpinetum. Most of recent woodlands established in the last 200 years represent Pino-Quercetum, Tilio-Carpinetum. As a rule, they are very poor in typical woodland herb species. Results prove that it is really long process of developing woodlands communities in recent woods. It is caused by very slow colonization new woodlands by forest plant species.

P2288. A Pilot Network of Plant Micro-Reserves in Western Crete

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Within the framework of a LIFE-NATURE-2004 project, a Pilot Plant Micro-Reserves Network is about to be established in Western Crete, Greece (Chania Prefecture). The network is comprised by 7 reserves selected among a total of 14 locations, within the boundaries of 3 pSCI (Natura 2000 sites): Gramvousa (GR4340001), Elafonisos (GR4340002), Lefka Ori (GR4340008). Each of the 6 reserves refers to each of the 6 plant species of Community priority (Directive 92/43/EEC) growing in Chania

Prefecture: **Androcymbium rechingeri*, **Anthemis glaberrima*, **Bupleurum kakiskalae*, **Cephalanthera cucullata*, **Hypericum aciferum*, **Nepeta sphaciotica*; the last reserve encloses part of the priority habitat type 9370, *Palm groves of *Phoenix*. The micro-reserves also embrace a significant number of other important plants (endemic, rare and threatened species). The network will be implemented by enclosures, wardening and on-site mild management measures. Permanent plots and automatic stations are to be established in each reserve for long-term monitoring. In addition, ex situ management of the targeted species and an information campaign will complement the network.

P2289. Can management compensate atmospheric nutrient deposition in heathland ecosystems?

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The increased deposition of nutrients from the atmosphere has contributed to widespread changes in both the structure and function of many heathland ecosystems throughout Europe. Therefore heathland management nowadays is considered as an important tool, to modify ecosystem impacts caused by atmospheric nutrient loads. In our study we compared the effect of mowing, prescribed burning, grazing and sod-cutting on the nutrient balance of heathland ecosystems. We analysed the atmospheric nutrient input, the output due to the removal of a particular compartment (biomass, organic layer, A-horizon), and increased leaching rates. Our results show that heathlands subjected to extensive measures will accumulate N on the long-term. Hence, N removal by means of mowing or prescribed burning cannot compensate atmospheric N deposition. As output/input relations for P exceed those for N, it is likely that N/P-ratios in the vegetation and soils on the long-term will increase. Hence, we assume that heathland ecosystems nowadays limited by N may shift to more P-limited ones on the long-term.

P2290. Modelling of the populations as a method of plant conservation ex situ.

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Among the specialists in Environmental Sciences are prevailed opinion about shedding the rare and endangered species of plants from plant cover under the influence of substitution of the natural forests by artificial. Our experience of modelling the populations of rare species of the plants in cultivated coenose in National Botanical Garden of Ukrainian Academy of Sciences does not confirmed this point of view. The rare species of Ukrainian flora (*Astrantia major*, *Crocus heuffelianus*, *Gymnospermium odessanum*, *Euonymus nana*, *Leucojum vernum*, *Lunaria rediviva*, *Scopolia carniolica* and other) and Caucasian flora (*Galanthus angustifolius*, *G. caucasica*, *G. woronowii*, *Erythronium caucasicum*, *Fritillaria caucasica* and other) were formed in 60 years old artificial beech, oak, hornbeam - oak, pine and spruce forests stable populations similar to the populations of these species in natural habitats.

The problem of the conservation rare plant species in artificial forest ecosystems is actual for all Eurasia. It's necessary to elaborate common programme of study, conservation and modelling the rare plant species population in artificial forests.

P2291. Regional landscape park "Dnieper islands" in Kiev. Its flora, vegetation, the tendency of synanthropization and measures of protection.

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The flora and the vegetation of the island in Kiev and a part of the Dnieper bank (together 6 000 ha) were learnt during 1999-2003. The significant floristic diversity was registered: 717 species, belonging to 356 genera and 99 families. But 40% of species are synanthropic (19% are alien species and 21% - apophytes). Nevertheless similar percentage was registered for other territories, even relatively well preserved, which reflects general tendency of synanthropization of the flora of Ukraine. Among the alien species epoecophytes and ergasiophytes prevail, 60 found species are expansive in the country. The analysis of flora shows its transitive character from forest to forest-steppe flora. The vegetation is represented by 15 cl., 24 ord., 33 all., 61 associations. Presence of 12 rare species and 5 plant communities

is noted. The protection of the territory in the category of the regional landscape park is desirable. This category exists since 10 years in Ukraine. It provides the combination of the nature protection, recreation and eco-education and is suitable for such a protected area within the capital city.

P2292. Vegetation and Flora of the Serpentine of Andaman Islands, India in need of protection.

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Typical vegetation and flora on the serpentine of the Andaman Islands, India, has not been included in the general description of the vegetation of these islands except by Parkinson (1923). Uniqueness of the vegetation and flora and the geomorphology of the particular areas deserve attention for prioritization in conservation. Serpentine soils occur sporadically in the Andaman Islands. Only one of these localities, Saddle hills in the North Andaman has been declared as a National Park. The present study describes the characteristic serpentine ecology, vegetation and flora of Saddle Hills and the Rutland Island, South Andaman. Remote sensing analyses help to discriminate typical serpentine vegetation from the surrounding non-serpentine areas. Sparseness, dwarfing and transformation to scrubby sclerophyllous vegetation with altitude all are evident. Besides, elevated concentrations of heavy metals like Ni, Co, Cr, and Zn, the soil match to other characteristics reported from elsewhere. Presence of a number of hyper-accumulators of these elements can be related to their endemism locally besides adding up to the list of Ni hyper accumulators.

P2293. Efficiency of the vascular plant protection for the south part of the Russian Far East in natural reserve net of Primorye and Priamurye

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The level of taxonomical diversity of the vascular plants in Primorye and Priamurye is very close to each other and counts 2592 and 2541 species of natural flora accordingly, but the degree of anthropogenic transformation of indigenous flora in Primorye is much above and reaches 22.1 % against 14.4 % in Priamurye (Kozhevnikov, Kozhevnikova, 2004). The Natural Reserve Net in Primorye and Priamurye consists of 15 Natural Reserves (NR). Total flora of 12 of them includes 1983 indigenous species and 240 adventive (alien) ones. Floristic researches in other 3 NR (Bastak, Bolonjisky and Botchinsky) are not finished yet.

The level of protection of indigenous flora in NR in Primorye makes 81.1 %, i.e. appreciably higher than in Priamurye (65.6 %); for Primorye and Priamurye this parameter as a whole is equal 76.8 %. The average adventive index of the flora of NR in Primorye and Priamurye makes 10.9 %. The high level of adventive index for NR of Priamurye is determined by Bolshehehtsirsky NR where it reaches the highest level (12.5 %) among all considered NR. As a whole, NR of Primorye differ from NR of Priamurye by higher level of presence of adventive species at their flora.

P2294. Long-Term Study on the Virgin Korean Pine-Broadleaved Forests of Ussuriyskiy Zapovednik in Southern Primorsky Kari, Russia

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The Ussuriyskiy Zapovednik is a nature reserve of the IUCN Category (Ia), with high biodiversity and complex stand structures located in Russian Far East. To understand the stand dynamics of the virgin Korean pine-broadleaved forests, two permanent study plots were established in 1954 and 1964, respectively. Plot 7 was established in 1964 (0.35ha) and plot 10 was established in 1954 (0.36ha). Both were re-measured in 1977, 1985, 1991 and 2003. Major tree species of the two plots were *Pinus koraiensis*, *Abies*, *Carpinus*, *Tilia*, *Acer*, *Ulmus* and *Juglans*. At times of plot establishment, there were 13 tree species in plots 7 (in 1964) and 11 species in plot 10 (in 1954). Tree number was highest in 1977 (777 trees/ha) in plot 7 and decreased gradually to 632 trees/ha by 2003. However, in the plot 10, there were 600 trees/ha in 1978, 520 trees/ha in 1991 and 555 trees/ha in 2003. Monitoring of species composition, stand density, stock volume and natural

regeneration characteristics at the study plots suggests that the dominant species, Pinus and Abies, will decrease because of low regeneration while hardwood species will rapidly increase and reach the canopy in the near future.

P2295. Relict and endemic plants of Kolkheti mires and their conservation

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Kolkheti lowlands, where the Sphagnum mires are situated belongs to: province - Colcheti or eastern euxinus, district - Colcheti lowland and foothills, world - oldest Mediterranean, region - submediterranean. The Kolkheti *Sphagnum* mires and its flora constitute relicts from the glacial period. Relict plant species which occurs mires of Kolkheti: *Sphagnum imbricatum*, *Sphanum papillosum*, *Drosera rotundifolia*, *Osmunda regalis*, *Carex lasiocarpa*, *Molinia litoralis*, *Rhynchospora caucasica*, *Rhynchospora alba*, *Lycopodiella inundata*, *Hibiscus ponticus*, *Cladium mariscus*, *Kostaletskia pentacarpa*, *Spiranthes amoena*, *Dactylorhiza majalis*, *Rhaphicarpa medwedewii*, *Trapa colchica*, *Trapa natans*, *Trapa maleevi*, *Salvinia natans*, *Nuphar lutea*, *Nymphaea colchica*, *Acorus calamus*, *Quercus imeretina*, *Quercus hartwissiana*, *Pterocarya pterocarpa*, *Buxus colchica*, *Pancratium maritimum*, *Asparagus litoralis*, *Solidago turfosa*, *Celtis australis* etc.

P2296. Persistence of exotic Australian fungi and genetic diversity of native fungi after eucalypt eradication in protected areas of the Iberian Peninsula

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The introduction of Australian ectomycorrhizal fungi with eucalypts increases eucalypt invasiveness in Iberian Spain. Spanish authorities, hence, promote the eradication of these exotic trees from National Parks. We study the persistence of the exotic fungi after the eucalypt eradication, and how this affects the populations of native fungi and the fitness of native trees we plant. We studied the fungal diversity in soils of former eucalypt plantations in which native trees were planted after eucalypt eradication. We used RFLP-T and DNA sequencing of the ITS regions of the nuclear rDNA for fungal identification in soil samples. Native species were the main fungi we found in soil of the plantations of cork oak (*Quercus suber*) (i.e. *Laccaria lacata*) we studied. Our genetic analyses indicated the populations of the fungal species colonizing the roots of native trees were very diverse (i.e. *Pisolithus* spp.). Many of the Australian fungi have disappeared from the assessed areas (after the eradication of the eucalypts), though some exotic fungi still persist (i.e. *Laccaria fraterna*).

P2297. Pteridophytes of the sandy coastal plains of Rio de Janeiro state, Brazil: species richness, distribution and conservation

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Restinga vegetation is found on sandy coastal plains in Brazil, and has strong links to the coastal Atlantic Forest. The most common plant communities are open scrub, beach-thicket, sedge swamp, creeping psammophytes, flooded forest and periodically flooded forest. A survey of pteridophytes found in the *restingas* of Rio de Janeiro is presented here, plus similarity indices between different *restingas* based on the pteridophyte flora. There are 19 families, 37 genera and 68 species; the following families are present: Aspleniaceae, Azollaceae, Blechnaceae, Cyatheaceae, Dennstaedtiaceae, Dryopteridaceae, Equisetaceae, Gleicheniaceae, Hymenophyllaceae, Lomariopsidaceae, Lycopodiaceae, Nephrolepsidaceae, Osmundaceae, Polypodiaceae, Pteridaceae, Salviniaceae, Schizaeaceae, Thelypteridaceae and Vittariaceae. The most species-rich families are Polypodiaceae (14 spp) and Pteridaceae (10 spp). *Polypodium triseriale* Sw. was found in all *restingas*. Similarity values are low for the pteridophyte floras of several *restingas* on the Rio de Janeiro coast. These results are important for effective conservation of the *restingas*.

P2298. Share of ancient forest plant species in recent forests as a criterion for their valuation and reconstruction: case study the Slowinski National Park (Poland)

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The Slowinski National Park (SNP), known as the World Biosphere Reserve, is situated in central part of the Polish Baltic Seashore. As result of authors' phytosociological investigations conducted there in 2002-2003 also the real and potential natural vegetation has been mapped. An analysis of both these maps revealed significant discrepancies between types of real and potential natural vegetation. Anthropogenic forest communities of substitution occupy large areas, especially in the southern part of SNP. Their affiliation to known associations of natural character is impossible since there are mainly monocultures of birches, spruce or different pine species, which partly have been established on formerly arable areas. An attempt of using the criterion of presence of ancient forest plant species for estimation of the degree of disturbance of each forest association is presented. This criterion was also used for elaboration of documents on valuation and reconstruction of phytocoenoses of chosen types, i.e.: *Betulo-Quercetum roboris*, *Quercu-Ulmetum* and *Stellario-Carpinetum*.

P2299. Conservation of an endangered shrub in the southeastern United States

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Pondberry [*Lindera melissifolia* (Walt) Blume, Lauraceae] is an endangered shrub that occurs in the southeast United States in seasonally flooded wetlands. Its distribution and abundance have been affected by habitat destruction and alteration. We investigated the establishment of new pondberry populations as an aid in conserving the species. In the fall of 2000 we dug equal numbers of young male and female pondberry stems from a natural population and planted them in pots. We then translocated them to five protected locations in Mississippi. Each pair of plants was surrounded by a wire cage on wooden supports, and was labeled. We watered the plants during dry spells for the first two years. We have monitored and measured the plants since they were introduced. After four years, pondberry survival has been good at three of the five sites. The Lower Mississippi Alluvial Valley, in which most present pondberry populations occur, is one of the most endangered ecosystems in the United States. Pondberry populations that die out usually will not be replaced. This study indicates that introduction of pondberry to new sites can be helpful in assuring survival of the species.

P2300. Vegetation of Altinbesik Cavern National Park (Ibradi-Akseki / Antalya-Turkey)

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Altinbesik Cavern National Park, where eco-tourism is very important, is located in the Western Taurus Mountains between the towns Ibradi and Akseki in the east and northeast of Antalya. WWF has identified in 1999 the 100 forest areas, which are the most valuable in terms of biodiversity in Europe and which must urgently be protected, and it has been pointed out that one of these areas, which are identified as "The Hot Spots of European Forests" and nine of which are found in Turkey, is the natural areas around Ibradi-Akseki.

Within the area 9 different kinds of communities belonging to the forest, maquis, water-side, rock and scree vegetations have been diagnosed, namely *Crataegus monogyna-Pinus brutia*, *Tordylium ketenoglui-Juniperus excelsa*, *Pistacia terebinthus-Quercus coccifera*, *Phillyrea latifolia-Arbutus andrachne*, *Nerium oleander-Platanus orientalis*, *Onosma frutescens base community*, *Arabis davisii-Alkanna oreodoxa*, *Bupleurum erubescens-Pelargonium endlicherianum*, *Geranium lucidum-Ricotia sinuata*.

Within the area, a clear dominance of *Quercetea ilicis*, *Quercetalia ilicis* and *Quercion calliprini* has been identified.

P2301. Important factors for the creation of nature reserves

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Since 1999, it is possible for local authorities in Sweden to initiate the creation of nature reserves in order to achieve a more locally founded conservation practice. So far 83 out of Sweden's 280 local authorities have taken advantage of this possibility and 169 nature reserves have been formed. The number of nature reserves is particularly low in local authority areas with dominating boreal forests which also often are the largest ones.

The reasons for these large differences between local authority areas are assessed. Primarily, the allocation of resources to the nature conservation authorities is an important factor. Further, it seems to be differences in attitude towards formation of nature reserves but also in views on how to evaluate different areas from a nature conservation viewpoint. These differences seem to be founded both on scientific studies or information as well as on less well founded prejudice.

Another important factor promoting the creation of nature reserves seems to be the presence of qualified and competent ecologists in the administration of the local authorities.

P2302. Conservation of threatened plants in the Cape Floral Region

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The Cape Floristic Region (CFR) has over 9000 species of plants. Recognized as a Global Biodiversity Hotspot, it currently has 526 threatened plant species.

Given the large number of threatened plants, and the limited capacity of conservation organizations, monitoring the status of populations of these species is extremely challenging. The South African National Biodiversity has set up a program that capitalizes on botanical amateur expertise. Volunteers survey fragments of threatened habitats for threatened plant populations, capturing abundance data, distribution data and threats affecting each species. These data are captured in a national database used for red listing and land-use planning. In addition, volunteers contribute to conserving critical sites for threatened plants through active management and awareness creation amongst landowners. Here we provide updated information on the threat status of plants in the Cape Floral region, explore the effectiveness of using volunteers for monitoring and look at the challenges of systematically surveying areas to ensure all populations are encountered for accurate threat status analyses.

P2303. Towards a Working List of Known Plant Species

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A gap analysis of the taxonomic coverage needed for an electronic Working List of Known Plant Species was carried out at a workshop organised by Species 2000 and the Royal Botanic Gardens, Kew, and sponsored by GBIF and the UK BBSRC. Creating such a list by 2010 is Target 1 of the CBD Global Strategy for Plant Conservation. Experts from around the world, and covering a wide range of taxa, carried out the analysis, based on existing checklist databases plus existing monographic and checklist activity, so as to identify the missing sectors which amount to approximately 45% of known plants. An attempt was made to identify contacts with groups of specialists who might assist with filling these gaps, as wide international participation will be needed if the target is to be met. The priority now is to organise and fund a progressive acceleration of activities, particularly for the missing groups. A further open 'satellite meeting' will be organised at the IBC after this session.

P2304. Global Patterns of Vascular Plant Diversity - where are the "most important areas for plant diversity"?

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The diversity of vascular plants is very unevenly distributed on the globe. We present world maps of species richness and endemism for vascular plants as well as different subgroups, such as gymnosperms. Most of the global maxima of plant diversity are located in mountainous regions within the humid tropics, where

suitable climatic conditions and high levels of geodiversity, i.e. the variety of abiotic conditions, coincide. Though the 20 global maxima of plant diversity (more than 3,000 vascular plant species per 10,000 km²) cover less than 7% of the land surface, they are inhabited by approximately 860 million people (about 14% of the human population). The questions to be addressed with our datasets are: 1) where are hotspots and good news areas for plant diversity, where are congruencies and differences to other global priority setting approaches 2) what are the factors causing the overlap between high diversity and high human impact, 3) is it possible to select a few indicators to assess the status of plant diversity?

P2305. The distribution of species of the tribe Neottieae (Orchidaceae) in Ukraine

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All species of the tribe Neottieae are rare in Ukraine and included in National Red book. The complete mapping of all known localities of species of the tribe Neottieae in Ukraine is especially important for the organisation of their conservation.

The tribe numbers 5 genera (Cephalanthera, Epipactis, Limodorum, Listera, Neottia) and 12 species. Epipactis helleborine, Listera ovata and Neottia nidus-avis are widespread in Ukraine. They occur in all forest and forest-steppe regions and the total amount of known localities is over 150. The species of genus Cephalanthera are widespread in West-bank of the Dnipro river part of Ukraine. Epipactis purpurata are found only in western and south-western regions of Ukraine. Only Crimean localities are known for Limodorum abortivum and Epipactis microphylla. The last species was found in Transcarpathia at the beginning of XXth century.

The species diversity and concentration of localities decrease from west to east that may be explained by ecological and cenotic peculiarities of species, the variety of antropogenous transformation of the territories and also different level of studing of regions.

P2306. Understanding a rare species. The case of *Orthotrichum rogeri* Brid. in the Pyrenees.

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Orthotrichum rogeri Brid. has previously been found in Austria, Belgium, Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, The Netherlands, Norway, Poland, Russia, Spain and Switzerland and it has been considered to be a rare moss throughout its range. Due to its reduced known distribution this epiphytic moss has been included in the Bern Convention (Appendix I) and Habitats Directive (Annex II).

Orthotrichum rogeri has often been confused with other species (*O. pallens* Brid., *O. stramineum* Hornsch. and *O. pumilum* Sw.) and it is possible that its area and, especially, its frequency will change with new studies. In a recent revision it has been possible to identify some new characters that allow a safer discrimination (Garillete, Lara & Mazimpaka, Nova Hedwigia 75 (1/2): 207-216. 2002).

The main aim of this project is to increase the existing knowledge on this species in order to contribute to the conservation of this taxon and its habitat. The study of its Pyrenean populations will permit to obtain information on its ecological requirements, reproductive biology, dispersion, demographic parameters, with the view of drawing its life history.

P2307. Bulbous plants and their protection in Azerbaijan

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Bulbous plants are biannual or long-term grasses on their vital forms. Representatives of bulbous plants in Azerbaijan belong to different families: *Alliaceae*, *Amaryllidaceae*, *Poaceae*, *Liliaceae*, *Iridiaceae*. Species of *Alliaceae* and *Liliaceae* are predominating. Bulbous plants widely spread in meadows, steppes, deserts, semi-deserts and the forest natural floristic complexes. They have mezophyte, xerophyte, mezoxerophyte and other ecological types. Presently, 120 species belonging to 12 genera (*Merendera* Ram., *Gagea* Salisb., *Allium* L., *Lilium* L., *Tulipa* L., *Muscari* Mill., *Galanthus* L., *Sternbergia* W.K., *Ixiolirion* Fisch., *Iris* L., *Gladiolus*

L., *Bellevalia* Lapeyr.) are under threat of extinction. Rare species (*Allium vavilovii*, *A. caespitosum*, *A. engenii*, *A. grande*, *A. microbulbum*, *A. panlii*, *A. pskemense*, *A. regelianum*, *A. sergii*, *A. trautvetteranum*) of these genera have been registered in the Red List of Azerbaijan.

P2308. The rare medicinal plants of the Ukrainian Carpathians under the conditions of introduction

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It is considered that the Ukrainian Carpathians are the treasure-house of medicinal plants. According to all available information the flora of this region has about 680 species of plants, which have the medicinal properties. The endemic, rare and disappearing medicinal plants are worthy of particular attention because it is important not only for biology but for the human health as the medicinal resources. According to The Red Book of Ukraine the rare and endemically plants in this region is counted about 89 species. The most important part of common strategy of the plant's care is their preservation under the introduction conditions. It is difficult, complex task which are solved by botanical gardens as well.

As a result of the introduction investigations is created the collection The Medicinal Plants that are counted about 250 species, which concern to 146 sorts and 48 families. The medicinal plants of the Ukrainian Carpathians in collection are 30 %, including plants from The Red Book: *Lilium martagon* L., *Lunaria rediviva* L., *Aster alpinus* L., *Coleficum autumnale* L., *Scopolia carniolica* Jacq., *Gentiana lutea* L., *Gentiana punctata* L., *Rhodiola rosea* L.

P2309. The rare species of flora Ukraine in Donetsk chain of hills

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Conservation of biodiversity natural ecosystems is one of the main modern problems. A Donetsk chain of hills is unique territory, where grows relic, endemic, rare and endangered species of plants. At the same time the industrial complex of Ukraine is concentrated on this territory with a plenty of the population. This considerably complicates conservation natural ecosystems.

In territory of the Donetsk chain of hills grows: 17 species included in Red Data Book of IUCN, 25 species included in the European Red List, 64 species included in the Red Data Book of Ukraine were established. Age and spatial structure of populations of the some rare species of plants (*Scutellaria cretica*, *Paeonia tenuifolia*, *Fritillaria ruthenica* and others) were investigated. Regularities of distribution, ecological and coenotical features rare species of flora of Ukraine on Donetsk chain of hills were determined.

On the basis of the received data principles of construction regional econet within the framework of National econet were developed.

P2310. Islands of the Baltic Sea as Refugia for Rare Plant Species

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Islands of the Baltic Sea being refugia of many rare plant species, requiring protection and monitoring, are the centre of attention of the scientific world on an international scale.

The detailed many years' floristic researches on the numerous islands of the Gulf of Finland let identify these islands as one of the most important plant areas (IPAs) in Europe, where effective protection, monitoring and management systems should be targeted. The flora of the islands characterizes by the following features: exceptional botanical richness, the presence of many Fennoscandia and Baltic Sea endemic plant species as well as a great number of species on the boundaries of their main distribution areas.

Due to specific nature features of the islands and high degree of island ecosystems' safety, their flora contains 130 species, listed as threatened in Red Data Books of Eastern Fennoscandia and the Baltic Region. Being highly vulnerable and sensitive to human

interference, the island ecosystems deserve special attention as highly unique and valuable oasis of virgin nature.

P2311. The Rare species of Vascular plants of Ukraine in flora. The Left bank of the Dnieper of Polissia.

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48 rare species of Vascular plants belong to 35 genera, 22 families and 3 sections, have been found out among the flora of the Left bank of the Dnieper of Polissia. 4 species of them are listed in the European Red List of Word - Wide Threatened Animals and Plants and 1 species are listed in the Red Book of International Union Nature Protection, 44 species are listed in the Red Book of Ukraine, 6 species are listed in the Convention on the Conservation of European Wildlife and Natural Habitats (Bern convention), 21 species are listed in the Convention on the International Trade of Endangered Species of Wild Fauna and Flora (CITES), 26 species are listed in the rare species of Vascular plants list of the Polissia.

Some rare species of Vascular plants in the Left bank of the Dnieper of Polissia such as *Urtica kioviensis*, *Rumex ucrainicus*, *Crategus ucrainica*, *Tragopogon ucrainicus* deserved special attention. All these 4 species are put Lawn on the European Red List of Word - Wide Threatened Animals and Plants, but on a national level they are not protected in Ukraine. To our mind, these rare species of plants is necessary to enter at in a new issue the Red Book of Ukraine.

P2312. Monitoring of medical plants in Russian Federation

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During last 25 years there was 10-15% decrease of *Vaccinium vitis-idaea* L. and *Arctostaphylos uva-ursi* L stocks because of ecotope destruction. The stocks of ruderal plants (*Achillea millefolium* L., *Valeriana officinalis* L., *Urtica dioica* L.) increased to 20-50%. Anthropogenic changes in flora caused the increase of *Tussilago farfara* L biological stock.

There was 20% diminution of *Hypericum perforatum* L. and *Origanum vulgare* L.bushes in 1980-1995. In some regions *Juniperus communis* fructiferous bushes vanished. The reason of populations state deterioration is over-pasture. At the end of 20th century bushes partly restored.

In 1985-1991 bushes of *Rosa acicularis* Lindl.and *R. majalis* Herrm.decreased to 8-12%. Since 1995-1997 in view of low level of agriculture on water-meadows they began to renew. By 2004 areas of *R. majalis* exceeded the level of 1983. Stock fluctuation of *Convallaria majalis* L. did not exceed 5%.Stock regression is more significant in central and southern part of Russia.

P2313. The Italian Seed Bank Network for native species conservation (RIBES)

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At the beginning of April 2004 a group of approximately 20 Italian institutions involved in seed conservation met in Trento (NE Italy) to discuss the possibility to team up to manage more efficiently native seed conservation practice at national level. The idea itself was launched by the authors of this presentation who are members of ENSCONET, the European Native Seed Conservation Network, and agreed to act at national level to provide a link between ENSCONET and the Italian setting. They received enthusiastic support from all institutions contacted. Since then further meetings took place in Rome, Milan and Pavia during 2004 and 2005. A consortium agreement, a charter, various internal regulations and an action plan were discussed in detail and approved with a participative approach. The poster presents and illustrates in detail this initiative that aims to contribute to the implementation of the Global Strategy for Plant Conservation at regional and national level (targets iii, viii, xiv and xvi).

P2314. Some biological aspects affecting *Mimosa* (Leguminosae-Mimosoideae) seedling establishment in Mexican natural ecosystems

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Seedling stage is an important phase in ecosystem natural regeneration. *Mimosa* species are well spread over Mexico and become dominant in disturbed ecosystems. This study focuses on seedling morphology and growth, and on seedling association with nitrogen fixing bacteria and arbuscular mycorrhizal fungi. Cotyledons are functional and persist during 6 weeks after germination. First eophyll is paripinnate and hypocotyl develops adventitious roots. In woody species, lignification occurs when seedlings are 4-6 weeks old. At that age, *Mimosa* seedlings also form nitrogen-fixing nodules and mycorrhizae. Mycorrhizal seedlings have more biomass than non-mycorrhizal ones, allocating more resources to the shoot than to the root; mycorrhizae improve seedling resistance to water stress and to predators, and favor soil nutrients uptake. The overall of these characteristics lead *Mimosa* seedlings to establish soon in time, becoming more competitive than those of other species. Morphological and growth features, plus symbiotic associations, may have a synergistic effect that could partially explain the ecological success of *Mimosa* species.

P2315. Study on the seed and seedling biology of nine highly endangered trees and shrubs endemic to the Caribbean: its implications for their conservation

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We compared the seed and seedling biology of nine highly endangered trees and shrubs endemic to the island of Puerto Rico: *Calyptronoma rivalis*, *Cornutia obovata*, *Crescentia portoricensis*, *Eugenia woodburyana*, *Goetzea elegans*, *Harrisia portoricensis*, *Juglans jamaicensis*, *Pleodendron macranthum*, and *Schoepfia arenaria*. They represent diverse critical habitats found on the island (humid and dry carst forest, coastal scrubs, dry serpentine forest, humid montane rainforest), as well as represent part of the taxonomic diversity of the plant species currently designated as endangered elements. Specifically, we assessed aspects of the flowering and fruiting of maternal plants, seed dormancy and germination, and seedling development in relation to their ecological requirements. Our results provide baseline data for in situ and ex situ conservation strategies of rare plant groups in the Caribbean, taking in consideration the differences in the species biology.

P2316. The Effects of Pre-Germination Treatments (GA₃, Polyamines KNO₃, H₂SO₄ and prechilling) of Endemic *Helleborus vesicarius* Aucher Seeds

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Some *Helleborus* species (*H. niger*, *H. foetidus*) have important biological activities and medicinal effects. *Helleborus vesicarius* Aucher is an endemic species and distribute in South Anatolia from Turkey. Germination of *H. vesicarius* seeds collected from Kahramanmaraş provinces during summer 2003 was evaluated using following treatments: control, concentrated H₂SO₄ and than soaked 400, 600, 1000 ppm GA₃ concentrations, spermidine at 0,01 between 1mM, spermine at 0,01 between 1 mM, GA₃ at 200-400 and 600 ppm and prechilling in 0 °C at 2 or 3 weeks. Germination was very low and first germinations were obtained after two months and germination trials were completed after 210 days. Germination ratio showed very differences ranging 0-80 % according to treatments. The highest germination 80% were obtained 200 ppm GA₃ concentrations. Higher GA₃ concentrations decreased germinations ratio. Spermine and spermidine at 0,1 mM and 0,01mM concentrations and stratification improved on germination.

P2317. In vitro propagation technique for conservation of two endangered *Dyckia* species (Bromeliaceae)

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A reliable method for *in vitro* propagation of two endangered species of *Dyckia* was described. *D. pseudococcinea*, found in the coastal sand dune known as Barra de Maricá, is suffering an increasing impact caused by urbanization that leads to a significant risk of extinction. *D. ursina*, endemic to Cadeia do Espinhaço, albeit occurring in a National Park area, is highly endangered because of many kinds of external interference such as erosive processes in tracks, collapses, landslides, extractive actions, garbage and pasture. The influence of media, explant type and genotype on morphogenesis was evaluated. The general regeneration pattern was similar in the two species. The regeneration frequency and shoot production were significantly higher in *D. ursina* as compared to *D. pseudococcinea*, in all treatments evaluated. Regeneration frequency and shoot production were more efficient from leaf explants in *D. ursina*, while best results were observed in response to stem-root axis in *D. pseudococcinea*. In the system described here high multiplication rates were obtained in response to low concentrations of BAP and NAA.

P2318. Conservation of zygotic embryonic axes of *Genipa americana* L. (Rubiaceae) using cryopreservation in liquid nitrogen.

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Genipa americana L. is threatened due to predatory collection of fruits and destruction of its habitats. *G. americana* has intermediate seeds, that tolerate partial water content reduction, but loose viability when exposed to subzero temperature. Therefore, conservation of its seeds using the conventional methodology is not possible and alternative methods are required. The objective of this work was to develop a cryopreservation protocol for *G. americana* axes. Seeds were desiccated over silica gel and cryopreserved in liquid nitrogen. Seeds were thawed and axes were excised and cultivated *in vitro* for viability assessment. Germination of axes excised from fully hydrated seeds (52% moisture content) was 97,5%. Desiccation reduced axes' viability and those excised from seeds containing 8% moisture content showed 44% germination. After cryopreservation, axes isolated from fully hydrated seeds (52% moisture content) presented 30% viability, but highest viability (100%) was attained by axes isolated from seeds with 8.1% moisture content. In conclusion, embryonic axes of *G. americana* can be cryopreserved with success just by adjusting their water content.

P2319. Seed Cryopreservation of Threatened and Protected in Ukraine Vascular Plant Species

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Long term conservation of plant genetic information within seed conservation as the element of plant reproduction is the main direction of the Global Strategy for Plant Biodiversity Conservation. The main role of the realization of the European Strategy Program of Plant Conservation is given to botanical gardens. The problem of genetic biodiversity preservation in up to date reality is the main in Ukraine as well.

In response to these challenges the Botanical Garden and the Center of Low Temperature of Lviv National University (Ukraine) began the development of national approach to conservation of genetic diversity of native plant species.

Seed conservation (in liquid nitrogen) protocols were developed for Ukrainian flora species (the rare species included). The experimental data showed the ability of method to be used in seed long term conservation with no genetic information losses.

P2320. Seed Morphology and Germination Variability of Three Native *Isatis* spp; *Isatis aucheri* Boiss (Endemic), *Isatis candolleana* Boiss (Endemic) and *Isatis glauca* Aucher ex.Boiss.

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Kahramanmaraş Sutcu Imam University, Kahramanmaraş, Turkey.

Isatis sp. produces indigo precursors using dye industries. Also some *Isatis* sp. are used antiviral, anticancer and anti-infective purposes. The genus *Isatis* comprises 26 species which are found in Turkey and 20 of these species are endemic. *Isatis aucheri*, *Isatis candolleana* which are endemic species and *Isatis glauca* distribute in South and Central Anatolia. These species collected from Kahramanmaraş provinces at different localities during July 2004. Some morphological characters about fruit and seed were investigated. The effect of some factors; prechilling, water soaked, Gibberellic acid (GA_3), KNO_3 , spermine and spermidine at constant and alternating temperatures on the break of seed dormancy in three *Isatis* seeds were studied. The highest germination ratio were obtained GA_3 treatment at all seeds. Higher germination ratio were obtained spermine and spermidine, water soaked, KNO_3 and prechilling treatments than control group.

P2321. Biodiversity in the deep-freezer - „Loki Schmidt Gene Bank“ for regional resources of wild plants

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There is growing awareness of the ecological, economic and cultural significance of wild plant species and their potential value as genetic resources. *Ex situ* collections like gene banks are necessary for conservation of genetic resources, for basic and advanced science and for clearing house effects. Botanical gardens should establish seed gene banks for wild plants for promoting integrated conservation efforts and for protection and conservation of our natural plant genetic resources. At the Botanical Garden of the University of Osnabrueck we established a gene bank for wild plants of Northwest Germany. We already collected material of 1300 accessions from 370 species and 65 families. The seeds are stored in minus 20 °C under dry conditions in special plastic bags. Each accession is listed in a database and is documented by herbarium specimens in OSBU. We have proposed a decentralised network of gene banks for wild plants together with other Botanical Gardens in Germany.

P2322. Issues on ESUs: Using molecular information for the *ex situ* preservation of genetic variation in plant populations

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While the preservation of genetic resources is emphasized by the most important international biodiversity directives, many *ex situ* conservation strategies still disregard genetic information or use its indications in a limited way. I propose a general sampling decision scheme that uses data for molecular markers to identify population groups that fulfill the definition of Evolutionary Significant Unit (ESU) with a view to maximise the amount of genetic variation captured for *ex situ* conservation. Comparisons among groups based on the values of F_{ST} are used to determine the significance of reproductive isolation and to define general sampling areas, and the probabilities of loss of rare alleles and the basic indicators of genetic polymorphism help quantify the importance of the genetic legacy and pinpoint the populations where intensive sampling should be carried out. This methodology eliminates a considerable amount of guesswork in the design of sampling priorities for *ex situ* conservation and it allows the implementation of non-genetic variables, thereby paving the way for a multi-disciplinary, conservation-driven integration of knowledge.

P2323. Setting up a DNA Bank for the Canarian Flora

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Despite the area of the Canary Islands is only about 5% that of mainland Spain, they host more than 50% of this country's plant endemicity and represent the most remarkable biodiversity hotspot in Europe and the northern quarter of Africa. Because ecological perturbations related to unsustainable human development are

outweighing the intrinsic survival assets of many Canarian endemics, a major challenge that we have to face today is the preservation of their genetic diversity out of its natural areas of distribution. In this contribution, we set forth the organisation and philosophy of the Canarian plant DNA bank at the Jardín Botánico Canario "Viera y Clavijo" to i) discuss several methodological issues associated with sample acquisition, processing and management, ii) provide a basic guide for conservation institutions that might be interested in developing a DNA bank for their regions' plant diversity, and iii) foster the establishment of scientific collaborations to invest our DNA funds in actions that lead to the improvement of the knowledge on the origins, diversification and conservation of the Canarian Flora through the use of molecular information.

P2324. Reproduction biology and *ex-situ* conservation of Caucasian endemic species *Dioscorea caucasica* Lipsky

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Dioscorea caucasica is a relict West Caucasian endemic of local distribution. It experiences severe human impact due to its medicinal properties. As the distribution range of the species is outside the present jurisdiction of Georgia, it is impossible to study *in-situ*.

We have done the first research on reproduction biology of *D. caucasica* in order to maintain the species. *Ex-situ* collections and seed bank were created.

Propagation capacity of *D. caucasica* was studied *ex-situ*.

The species is dioecious. Generative structures are fertile. Pollen fertility is 84%; 77% germinates in artificial medium. Seed-buds are fertile; fertilization and embryogenesis proceed normally. Potential seed production capacity is 6 per flower; actual quantity of seeds is high (4), which indicates high reproductive index of the species. Proportion of seed germination and sprouting is 76-78%. Seedlings develop well; their majority is male.

It was determined, that *D. caucasica* has an actual capacity to produce viable seeds and offspring; however, pronounced deficiency of female plants along with other factors (anthropogenic etc.) causes the decline of populations.

P2325. Tools for identifying seed desiccation sensitivity: where climate meets seed biology

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For the majority of seed plants (> 92%), the ability of their seeds to tolerate desiccation is a key adaptive trait. However, the remaining c. 8% of species with recalcitrant (desiccation-sensitive) seeds, pose a significant challenge for *ex situ* conservation. In addition, because this group contains many heavily utilised tree species of socio-economic importance it is of significant conservation importance. Here, in line with Target 3 of the Global Strategy for Plant Conservation we present two tools for the identification of species with desiccation-sensitive seeds. Firstly, we have developed a desiccation screen that enables the rapid identification of desiccation-sensitivity using small quantities of germplasm (100 seeds), which we will illustrate using examples from the Palm family. Secondly, for tree species we present a globally applicable model for predicting, based on seed and plant habitat variables, the probability of seed desiccation sensitivity. This model is based on species from four continents and a variety of climatic zones: savannah / dry-land, temperate woodland, tropical semi-deciduous and wet forest.

P2326. Arboretum Dona Ines: an *ex situ* conservationist paradise in the Caribbean

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Counting with 12-15,000 plant species, the West Indies are one of the richest centres of evolution and ecological diversity of the neotropics.

25% of its flora is restricted to the Caribbean area, and 10% of its endemics are facing extinction. *In situ* conservation does not cover more than 7% of the Caribbean land, thus *ex situ* approach is a need.

The arboretum Parque Doña Inés of San Juan, Puerto Rico, constitutes the first serious attempt to develop a conservation

collection within the Caribbean region. Since the year 2000 the Park is collecting germplasm based on concepts of infraspecific diversity in order to maximize the representation of each taxon's eco-geographical and genetic diversity. The arboretum recognizes its regional responsibility in maintaining, propagating and making available stocks of critically threatened species for scientific research and eventual reintroduction in habitat. It also recognizes itself as an institution capable to raise environmental awareness for the West Indian's magnificent biodiversity, and promoting appreciation for the Caribbean's distinctive plant and animal life.

P2327. The role of seed science in the GSPC

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The 'terms and technical rationale' for the targets of the GSPC articulate the importance of science to evidence-based decision making and delivery of conservation objectives. For the *ex situ* conservation of plants as seeds, there are five main targets (3, 8, 9, 10 and 15) our seed science has addressed. We have given prominence to recalcitrant seeds studies (Target 8) by developing two enabling methodologies (Target 3): a predictive model for encountering desiccation sensitive seeds and a protocol for the rapid assessment of seed desiccation tolerance. Currently we are developing practical conservation methods for recalcitrant seeds using cryopreservation (Target 8). Reflecting the goals of Targets 9 and 15, we have established across 14 countries a Darwin Initiative seed research network for studies on 60 tree species of value for sub-Saharan Africa. Finally, we are assessing the contribution of seed traits towards the competitive advantage of a range of invasive plant species in support of the development of management plans (Target 10).

P2328. Seed cryostorage of endangered plants in Poland

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Among 2.750 taxa of Polish vascular flora 418 species are threatened with extinction. Therefore several projects were carried on *ex situ* conservation by the botanical gardens in Poland. In 1992 in the Botanical Garden of the Polish Academy of Sciences in Warsaw it was organized the National Seed Bank for the long-term seed storage of the Polish native plants with special emphasis to Red List of Endangered Plants and plants legally protected by law. For seed preservation the storage in cryogenic conditions - in the vapor of liquid nitrogen at temperature ca. -160°C was chosen. Because of seed dormancy problems the special protocols for seed germination for each particular plant species were prepared. Also for each species the seed tolerance for ultra-low freezing was tested. Presently in cryogenic seed bank are preserved 78 species represented by 280 populations. Thanks to seeds preserved in the cryogenic seed bank the successful reintroduction into primary localities was done for 4 extinct in the wild or critically endangered plants species in Poland. Presently the investigations are carried on within the European Union framework project ENSCONET.

P2329. Ex situ Conservation: an Integrated Approach in the Use of a Living Collection

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In the Ural Botanical Gardens of the Academy of Science of Russia, in Yekaterinburg, there is a unique collection of species from the genus *Salix* L. There are more than 2000 accessions, including 147 species from different regions of the world, 136 hybrids and 29 forms. This collection is used, not only for taxonomical and other scientific studies and for educational purposes, but also in an extensive breeding programme, which is directed at several applications: landscape-gardening, basketry and furniture production, re-vegetation and bioremediation, manipulating tannin content and increasing biomass production through short rotation coppicing. In the collection, there is a large number of accessions that combine several useful properties (characteristics) and therefore may have great potential in multipurpose breeding programmes. These characters were analysed and correlated with the taxonomy, biogeography and habitat of the accessions.

P2330. Ex-situ conservation of *Liparis loeselii* (Orchidaceae) at Eotvos Lorand University, Hungary

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The fen orchid *Liparis loeselii* (L.) Rich. was formerly known from over 8 localities in Hungary, but now occurs only in four sites, which all are very vulnerable. Surveying conservation possibilities, selected European populations of *L. loeselii* were studied by three molecular markers (two plastid spacers and nuclear ribosomal ITS) in order to reveal the origin and the genetic connections of Hungarian populations. In addition, asymbiotic and symbiotic germination of *L. loeselii* were studied. Germination array was tested on different asymbiotic media (different macroelement and organic nitrogen levels) in dark and light with and without cytokinin. For symbiotic germination and growing of asymbiotically germinated seedlings with fungal partners, symbiotic fungi were isolated using different methods. "Root segment technique" was proved to be less effective than "in-situ protocorm trap" method by which symbiotic fungal partners could be isolated more successfully and causes less disturb in natural populations. Fungal partners were identified by the DNA-based method ITS sequence analysis.

P2331. Micropropagation of *Gentiana lutea* L. and *Arnica montana* L.

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Gentiana lutea L. (yellow gentian) and *Arnica montana* L. (arnica) are medicinal plants species that should be protected by law as being overcollected. They contain valuable biologically active substances and are wide used in medicine and pharmaceutical industry. The purpose of this investigation was to elaborate efficient methods for *in vitro* propagation of both species. The explants most suitable for propagation are already identified i.e. stem segments with an axillary bud from plants that have germinated *in vitro*. The optimum nutrient media for germination, propagation and rooting *in vitro* of *Gentiana lutea* and *Arnica montana* are also characterized.

The results will contribute to the protection of both plant species and to the application of their perspective forms to produce ecologically pure products as crude matter for the industry and prevention of certain diseases.

P2332. Use of AMF in active protection of *Plantago atrata* and *Senecio umbrosus* - endangered plant species from the Tatra Mts.

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The flora of the Tatra Mts. is extremely rich in rare plant species. Among them, *Plantago atrata* Hoppe and *Senecio umbrosus* Waldst. & Kit. are seriously endangered. The first species is included in the world list of threatened plants and the second is extinct in the wild in Poland. Both species were found to be strongly mycorrhizal and dependent on mycorrhiza, while cultivated under laboratory conditions. The more intensive growth of mycorrhizal plants in comparison to non-mycorrhizal ones was clearly shown using diverse AMF strains. The inoculation with mixtures of strains was shown to be the most effective in plant growth stimulation. Due to the risk of plant destruction in natural sites, the investigations in the field were limited to studies carried out on arbuscular fungi that occurred in the vicinity of plant roots. AMF spores were identified and trap cultures were obtained. The strains obtained will be used for the inoculation of plant material multiplied and reintroduced into the experimental plots. Plants should be equipped with fungi suitable for multiplication under greenhouse conditions and for reintroduction into natural sites.

P2333. Mexican bamboos: *ex situ* conservation and research

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Mexico is represented by eight genera and 35 woody bamboo species which include 12 endemic species. They are found in the rain forest and tropical regions of Mexico from sea level to 3000 m altitude. Information on mexican bamboos is scarce and they are

almost unknown. A collaboration was made between INECOL, ITC and BOTA to start a long term project "The Native Bamboos of Mexico" with a main objective to create the national bamboo collection in the botanical garden and herbarium of INECOL. These collections will be the basis for multidisciplinary bamboo studies and will be the first *ex situ* bamboo conservation collection in Latin American. Since two years of this project 60% of the species are represented *ex situ* with their respective herbarium vouchers. Two studies were completed, Vegetative propagation of *Rhipidocladum racemiflorum* and Bamboo uses in Monte Blanco. Preliminary results have been reported in national and international congress, seminars, bamboo magazine, shorth communications as well as regional and national exhibitions. Studies in progress are conservation of endemic species, systematics, data base of uses and insects associated with bamboos.

P2334. DNA Banks: Another tool for the conservation and sustainable use of plant genetic resources

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The World has an enormous amount of biodiversity that is largely unexplored, misunderstood and not adequately documented. A great deal of attention is being paid to the fate of natural resources and the potential for loss of biodiversity. The conservation of plant genetic resources is important and it is essential that many diverse methods are adopted including maintenance in natural conditions and representation in *ex-situ* collections. DNA Banks store extracted plant genomic DNA which is made available to researchers for phylogenetics, phylogeographics, population studies, gene discovery and marker development. The Australian Plant DNA Bank is an example of a plant DNA Bank which aims to preserve representative genetic information from the Australian flora and cultivated species. Information regarding the species in this collection is available via the World Wide Web (www.dnabank.com.au). The operations, technical issues, and benefits of a plant DNA bank will be discussed.

P2335. Virus field-infection in *Allium* and successful elimination of viruses in the Gatersleben Genebank

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Viruses impose a common problem on the cultivation of vegetatively propagated alliums in the field and are a risk for the maintenance of genetic resources. The Gatersleben Genebank comprises a large collection of vegetatively maintained accessions like garlic, shallot, top onion, wild *Allium* species and hybrids. Virus screening tests were performed on leaves, bulbils and cloves from these materials. The occurrence of 6 viruses was recorded by means of virus identification based on ELISA-tests and tissue print immunoassay. All field accessions of garlic and shallot were infected with several viruses. All tested parts of a plant had a similar virus status. Wild alliums were specifically infected with viruses. Investigations were done to eliminate the viruses in garlic and shallots via meristem culture, thermo- and chemotherapy with the aim to establish virus-free clones *in vitro*. Virus elimination through meristem culture was successful by using small meristems. Thermo- and chemotherapy increased the elimination rate up to 80%. The efficiency of virus elimination was depending on the specific virus. Latent viruses were permanent and hard to eliminate.

P2336. From laboratory to field: the use of material from an *ex situ* collection in the introduction of a rare moss

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The Micropropagation Unit at the Royal Botanic Gardens, Kew, has been involved in the *ex situ* conservation of bryophytes since 2000, with a unique project funded by the UK statutory conservation agencies. The project involves the collection, culture, cryopreservation and introduction of rare UK bryophytes. Material is in culture for six British populations of the European endangered moss *Orthodontium gracile* Schwägr. ex Bruch, Schimp. & W. Gümbel. Protonemata of *O. gracile* have been successfully cryopreserved and are in long-term storage. Cryopreserved material has been recovered and weaned onto sandstone substratum. A preliminary introduction of this material was

undertaken at the Kew managed estate, Wakehurst Place, Sussex, UK in early 2005 using material collected from the vicinity. Survival, growth and development of the moss were monitored initially and continue to be observed. Preliminary data from the introduction are presented. Future development and global application of the methods are discussed.

P2337. Macromycetes of diverse plant communities: conservation *ex situ*

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Conservation *ex situ* of macromycetes of various ecological and taxonomical groups is a promising aspect of fungal conservation. Macromycetes diversity is preserved and maintained in pure culture in the LE (BIN) Culture Collection. Biota of macromycetes mostly has a direct correlation with plant communities in its ecotope. Macromycetes from deciduous, coniferous and subtropical forests, grassland communities, arctic tundra and alpine zones were cultured for conservation *ex situ*. Over 10 natural reserves and national parks of Russia were studied in this regard. It was shown that macromycetes of different taxonomical, ecological and trophic groups have different potentiality for growing and maintaining in culture. Ectomycorrhizal fungi were very complex for culturing and conservation *ex situ*. Most saprotrophic and especially xylophilic fungi could be easily isolated and maintained in culture. However culturing of fungi from certain genera of these groups caused problems. Understanding of macromycetes and plants correlation makes the work on macromycetes conservation *ex situ* more productive.

P2338. Bryophyte conservation in Romania. History and Perspectives

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Historically, bryophytes are not as well conserved as phanerogams, mainly because of their size and weak economical importance. Also, there are very few bryologists and the education on this topic is poor.

Bryophytes are primarily conserved in nature reserves or national parks, indirectly, but the instigation of Phanerogams conservation permitted the development of bryological studies in some protected areas.

From 1990, the following actions have been taken: (1) publication of comprehensive review papers (including frequency lists, chorology of Romanian mosses, red lists); (2) implementation of international legislation (Bern Convention, Habitats Directive) requested for a better identification of areas proposed for Nature 2000 network, as an EU demand for candidate countries and (3) project development (Bioplatform, Important Plant Areas).

Bryophyte conservation strategy is focussed on identifying the most important sites and habitats for bryophytes; providing educational resources on bryophytes, especially for students, foresters, national parks staff, etc. which will be involved in monitoring of some species (e.g. *Buxbaumia viridis*).

P2339. The native and exotic plants of Samarkand and their protection

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It is discussed on endemic and exotic plant species of Samarkand region of Uzbekistan. The local flora of Samarkand region consists of about 4500 different species, most of them are considered as a valuable medicinal, aromatic, industrial and ethnobotanical plants. Most of these plants can be also a valuable source for industrial production of different products and they also can be used for ornamental landscaping purpose as well.

At present day, most plant species are in endangered situation. Due to different factors, mostly anthropogenic one, the area of distribution of endemic and exotic plants as well as their biomass are degrading day after day.

It is propose different ways of protection of these plants. Long-term field and laboratory experiments showed promising technologies for their cultivation and introduction in a botanical gardens, temporarily protected areas as well as in a nurseries of educational establishments. Two special nurseries were established in the territory of forestry service of Urgut and Nurobod districts of

Samarkand region of Uzbekistan. Seedlings of endemic and exotic plants were successfully transplanted to other places.

P2340. In vitro and ex situ culture of hepatics- an innovative approach for an inventory

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The canton of Geneva has a rich flora due to its wide diversity of micro-habitats. However, its hepatics have not been studied for over a century. The canton has undergone a large amount of environmental changes and urbanisation during this period. In the light of this, an inventory of hepatics is overdue. We now know that the rare thalloid hepatic *Riella reuteri* Mont. is extinct in Geneva because its specialised aquatic habitat has been destroyed. An inventory, using traditional techniques and the innovative methods of ex situ culture (soil, wood, sand), provides a complete list of species presence in the specified area in terms of the above ground flora and the soil bank diaspores. We present the results of our soil culture experiments which have revealed some species that are only present in the soil diaspore bank, such as *Anthoceros agrestis* Paton, a species new for Geneva. In terms of conservation, a completed inventory permits us to assess conservation risks for different species, dependant on the different habitats and soils. By using in vitro culture techniques we can propagate material for future re-introduction trials in the canton.

P2341. Cold adaptation as factor to increase re-growth efficiency in cryopreservation of vegetatively propagated germplasm in genebanks

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Living plant collections are faced by problems to safely keep germplasm of which no seeds can be stored. This is important for many vegetatively propagated crop plants. Cryopreservation is the most effective and safest method to maintain germplasm for long time. Although the general usability of this method has already been demonstrated for many species, there are still genotypes of low response in most of them. The Gatersleben genebank holds germplasm of potato (more than 1000 accessions), garlic and mint in cryopreservation. In all these species, low temperature pretreatments support the cryopreservation effect by increasing the re-growth rates. This stimulation is present irrespective of the used methods (vitrification, droplet freezing or combination of both) in potato. In garlic, alternating temperature regimes act stronger than permanent low temperature. Alternating temperatures are also effective in mint. Cold adaptation and the underlying physiological processes are discussed in general with respect to their use for germplasm storage. Furthermore, general aspects of the cryopreservation technology in the IPK genebank are summarized.

P2342. The Use of Herbarium Resources to Improve the Targeting of Seed Collection Programmes for Ex Situ Conservation

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Target 8 of the Global Strategy for Plant Conservation prioritises collection of threatened species for ex situ conservation. This requires careful planning at the front end of conservation programmes. The MSBP is bringing together local experts and RBG Kew specialist staff to meet this challenge. The team are using information from herbarium specimens and literature to prioritise species for conservation, and produce collection guides. We extract, database and geo-reference information from both the Kew and Paris (Francophone partners only) Herbaria. When available, we combine this with data from the Herbaria of the country where collections are being made. The data is then subjected to geographical information systems analysis to provide i) recorded distributions; ii) preliminary assessments of extinction risk. In parallel with the data extraction, digital images of herbarium specimens are taken to provide partners with visual tools for rapid identification of target species in the field. So far 11 collection guides have been produced for six countries. The difficulties in this approach and the benefits of its products will be outlined.

P2343. Asymbiotic germination of *Dendrobium aqueum* Lindl. - a rare and endemic orchid of India.

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An efficient protocol for a rapid multiplication of *Dendrobium aqueum* Lindl., a rare and endemic orchid species of southern India, was made through asymbiotic germination method. The cultural requirements for *in vitro* seed germination, protocorm growth and seedling proliferation have been studied. The percentage of seed germination on different media compositions viz., modified Knudson C, Phytamax and Murashige and Skoog, enriched with the addition of 10% coconut milk, was ranged between 80 and 95%. Among the three media compositions used, modified Knudson C supplemented with 10% coconut milk was found to be more effective in terms of number of protocorm like bodies (PLBs) and shoot proliferation. The observations made on the effect of various complex organic additives showed that the banana pulp was more effective followed by coconut milk and citrus juice. Root formation was observed in 10 to 20% of the shoots cultured either with banana pulp or coconut milk. The root induction was otherwise achieved with modified Knudson C basal medium without added supplements. More than 70% of the seedlings were successfully established in the community pots.

P2344. Ecology of *Carica candicans* A. Gray (Caricaceae) in four hills of Lima - Peru

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Carica candicans "mito" is a plant that can live under low water conditions. Nevertheless, excess of leaf eating for herbivorous and a deficient regeneration because of unsuccessful germination in natural conditions, has put to *C. candicans* in risk of extinction. The present work was carried out in four of the main hills in Lima - Perú, Lachay hills, Amancaes hills, Carabayllo hills, Villa Maria hills. In all of these hills pH and electric conductivity of soils were ranging 4 - 7.8 and 0.61 - 1.33 mS, respectively. Moreover, it was evident a little number of individuals of *C. candicans*. So, in Lachay hills exist 154 individuals (37 female and 117 male), in Amancaes hills exist 4 (3 female and 1 male), in Carabayllo hills exist 5 (all male), Villa Maria hills exist 11 (4 female and 7 male), all of them associated with rocks, stones and shrubs and herbaceous vegetation. We registered biometric data of fruits, ranging length 50 - 168 mm, width 40 - 60 mm and weigh 50 - 200 g, number of seeds 75 - 190. We collected fruits in order to maintain their seeds for regeneration works, so 29 months (880days) after sow first plants are flowering.

P2345. Conservation study of endangered *Potamogeton* species in Japan: DNA sequence data allow the differentiation of a hybrid.

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The destruction of wetland environments in recent years has been extensive, and as a result of this habitat loss many aquatic species are in danger of extinction. For conservation purposes, this study used molecular data to investigate Japanese species of *Potamogeton*. *Potamogeton x inbaensis* is considered a hybrid between *P. lucens* var. *teganumensis*, which is classified as critically endangered, and *P. malaianus*. Typical individuals that are intermediate in morphology and leaf petiole length are usually used for identification. In the field, however, the variable leaf morphology of these three *Potamogeton* species makes it more difficult to identify them. We used sequences of the internal transcribed spacers of the nuclear ribosomal DNA (ITS) and a non-coding spacer between *atpB* and *rbcL* on the chloroplast DNA to reveal their interspecific genetic relationships. Investigation showed that leaf morphology and stem anatomy are not suitable for identification purposes; in the future, therefore, we will rely on molecular data.

P2346. Ex situ conservation in the Balkan Botanic Garden of Kroussia (N Greece): the Balkan endemic *Cerastium decalvans* Schlosser & Vuk.

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Cerastium decalvans, as a Balkan endemic not cultivated in Botanic Gardens, falls into the plant conservation priorities of the Balkan Botanic Garden of Kroussia (BBGK). Its cultivation in BBGK offers the potential of future re-introduction in case of decline of its natural populations. Wild growing individuals were collected at forest clearings of Mt Vermion (NC Greece) at c. 2000m. Although flowers appeared at sea level, no seed production has been observed yet at the mother plantations. Rooting of leaf cuttings has been studied using various concentrations of auxins at different seasons of the year. Optimal success was obtained in autumn, 19 days after they were treated with 4000 ppm indole-3-butyric acid (IBA), in peat: perlite (1:3) mixture, under mist. Young plants were transplanted at trays, where they remained for 45 days. Survival percentage exceeded 90% and plants were re-transplanted in 2.5 liter pots while growth and development of the plants were monitored for 45 days. Plants were finally established in the BBGK at c. 600m. Almost all the laxly caespitose plants survived both the dry summer season and the winter cold period but no flowering appeared yet.

P2347. Phenology of *Ismene amancaes* (r&p) Herbert. (Amaryllidaceae) and feasibility of its reintroduction in hills of Lima - Perú

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Human activities have put to *Ismene amancaes* (R&P) Herbert "Amancaes" in vulnerable condition. The objective of this work was to evaluate the biological cycle of "amancaes" and feasibility of its reintroduction in hills of Lima - Perú. We evaluated this specie in Villa Maria Hills (12° 08'11" LS-76° 55' 27" LW) at 588 m over sea level (m.o.s.l.); and we estimated the age of bulbs observing thickness of outer dry cataphylls and diameter of bulbs, also we registered phenology of this specie during 2003 and 2004. *I. amancaes* grows from 535 m.o.s.l. to 750 m.o.s.l. where we can find it flowering during winter months but disperse. Collected seeds were sowed in trays and maintained at 26°C±2° in darkness until a little bulb rise, then a standard substrate (Promix B5) was added observing a good growth of bulbs. The biological cycle of this specie is limited for high temperatures, soil humidity and changing environmental conditions, resulting in a later and accelerated growth during 2003, and earlier and wide growth during 2004.

P2348. Conservation efforts over native flora in Central Chile

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The area located between 25° - 40°S in central Chile presents a Mediterranean climate characterized by cold and rainy winters and variable period of drought in summer. The natural vegetation in this area was disappeared principally by road constructions, felling of trees and shrubs for industry and energy, agriculture, overgrazing, fires and mining working. O'Higgins region (33° - 35°01'S - 70°W) has a principal economic activities the fruit culture for exportation, wine production and mining exploitation, with human perturbation beginning in XVI century.

Notwithstanding, this region its present north limit of several species, endemisms very endangered as *Neoporteria horrida* var *aspillagae* (Cactaceae), *Avellanita bustillosii* (Euphorbiaceae), *Beilschmiedia berteroaana* (Lauraceae), *Leucocoryne codehuensis* (Alliaceae), and so on.

During this year CONAF and Regional Government began a project "Red Book of terrestrial flora and fauna, and interesting site for conservation of region del Libertador Bernardo O'Higgins", and collecting seeds and other material for propagation of endangered species.

P2349. Botanical-geographical diversity of the calcareous mountains in West Transcaucasus (Colchis, Georgia)

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In Georgia calcareous biotopes occur only in its west part: Abkhazia, Samegrelo, Svaneti (not very common), Racha-Lechkhumi, Imereti. Partitioned relief, karst forms and canyons feature the calcareous ranges in the gorges of rivers Bzifi, Okumi, Enguri, Chanistskali, Khobi, Tekhuri, Tskhenistskali, Rioni.

Many plant species specific to calcareous habitats are regional Colchic endemics and relicts: *Thymus ladjanuricus*, *Galanthus schaeoricus*, *Asperula kemulariae*, *Muscari alpanicum*, (Racha-Lechkhumi); *Potentilla kemulariae*, *Kemulariella colchica*, *Allium albobianum*, *Betula megrelica* (Samegrelo); *Campanula kemulariae*, *C. irinae*, *Veronica galathica*, *V. imeretica*, *Scrophularia imeretica*, *Aquilegia colchica* (Imereti); *Campanula mirabilis*, *Arabis sachociana*, *Pimpinella idea*, *Gentiana paradoxa*, *Aquilegia gegica* (Abkhazia).

Colchic understory (*Laurocerasus officinalis*, *Rhododendron ponticum*, *Rhamnus imeretica*, *Ilex colchica*, *Vaccinium arctostaphylos*) and *Buxus colchica* forests with *Staphylea colchica* in the gorge of the river Tsachkhuri (Samegrelo) create unique landscapes in the botanico-geographic province of Colchis.

P2350. Genetic Resources of Native and Wild Ornamental Bulbous Plants in Hyrcanian Region of Iran

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Genetic variability is the base of variation in ornamental plants. Hyrcanian region in north of Iran has high plant diversity because the topographic, edaphic and climatic conditions is very variate. This research was conducted to morphological and ecological studying and introduction important wild bulbous plants in this region such as *Lilium ledeburii*, *fritillaria spp.*, *Tulipa spp.*, *Gagea spp.*, *Bellevalia spp.*, *Ornihtogalum spp.*, *Scillia spp.*, *Allium spp.*, *Erihronium denscansis*, *Muscari spp.*, *Leucojum aestivum*, *Galanthus nivalis*, *Sternbergia spp.*, *Narcissus spp.*, *Iris spp.*, *Crocus spp.*, *Gladiolus spp.*, *Colchicum spp.*, etc. Some of the plants are ornamentally used but their wild relatives are valuable as genetic resources. Also Some of them such as *Lilium ledeburii* is exposing to endangering and genetic erosion due to human activity, natural damages and cultural practices. Thus in respect to valuability of gene pool for breeding programs including compatibility to unfavorable conditions and introducing new cultivars it is necessary that organisations and research institutes try to protect these natural resources from destroy and endangering.

P2351. First approach to the biology of *Polygala vayredae* Costa (*Polygalaceae*), an endemic plant from Oriental Pyrenees

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There is a consensus that studies on the reproductive biology of endangered, rare or threatened species may be useful for understanding why they are endangered, rare or threatened. Information obtained from such studies may be useful for evaluating alternative *in situ* and *ex situ* management strategies. *Polygala vayredae* Costa is an endemic species from oriental Pyrenees and a relic that survived to the glaciation's period in the Tertiary, perhaps because it refuged in the enclave where it actually occurs. This species is poorly known, and because of its restricted distribution area it is protected and very important for conservation. In the present study, we investigated aspects of the reproductive cycle of *P. vayredae*. For that, morphological and functional characteristics of flower and pollen source were analysed as they have major effects on the reproductive success. The first approach gives us information about the reproductive system of this species. With this information we intend to increase the knowledge on the reproductive biology of this species and provide relevant know-how that is essential for a correct management and conservation of *P. vayredae*.

P2352. Ecology and management of an endemic subspecies - *Tephrosieris longifolia* subsp. *moravica*

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Tephrosieris longifolia subsp. *moravica* is a critically endangered taxon endemic of the Western Carpathians with a restricted

geographical distribution on the border of Slovak and Czech Republic. Since it is known, it occurred only on restricted number of localities, where it survived almost exclusively in small populations. Taxon occurrence is restricted to a very specific vulnerable ecotonal habitats. Knowledge of both the taxon life cycle and its ecological requirements is still insufficient. Recently, decreasing taxon abundance and reducing number of flowering individuals was observed which could be a consequence of unfavorable weather excesses as well as unsuitable agricultural practices during the last centuries.

The detailed analysis of plant communities with the taxon occurrence supported by the analysis of relevant environmental factors is an attempt to estimate the main ecological characteristics of the taxon. The results of recent censuses are evaluated and the perspectives of maintaining the viable populations are sketched. Necessary recommendations of suitable management types are offered and some notes are made to the monitoring methods.

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P2353. Seed conservation activities for narrow endemic species in the Italian Alps

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From 2002 onwards four *ex situ* seed conservation projects for endangered and narrow endemic alpine species were devised and put in operation independently in four distinct geographical districts of the Italian Alps, being based in Chiusa Pesio (southern Piedmont, western Alps) Pavia (Lombardy, central Alps) Trento (Trentino, central-eastern Alps) and Padua (Veneto, eastern Alps). These initiatives were supported by a very diverse range of funds and local expertise, including volunteer work, yet they aimed to serve the same purpose and employed roughly the same consolidated methodology. Highlights of the target species selected, include the three alpine endemic genera *Berardia*, *Physoplexis* and *Rhizobotrya*, and many point endemics such as *Viola comollia*, *Daphne reichsteinii*, *Callianthemum kernerianum*, *Campanula petraea*, *Moltkia suffruticosa* etc. In the context of the newly born Italian seed bank network these projects will act in a co-ordinate way to provide a regional unit within the Italian network. Future perspectives would naturally extend the conservation action to the alpine bio-geographical region seeking trans-boundary partners.

P2354. Reproductive Biology of *Goetzea elegans* Wydler, an endangered Caribbean tree

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Endangered species commonly confront constraints in their reproductive biology. However, although knowledge of these processes are of primary importance for implementing sound conservation measurements, little data is available for most endangered species of the Caribbean islands. *Goetzea elegans*, an endangered tree endemic to the island of Puerto Rico, represents an illustrative case study on rare plant conservation in the region. The species is known from little over a hundred reproductive individuals, whose flowers are visited by vertebrates and invertebrates, including native and invasive species. We studied the reproductive biology of *Goetzea elegans* in order to assess implications for its conservation. Specifically, we determined the breeding systems of the species by experimental flower manipulations and compared pollination efficiency between the invasive bee *Apis mellifera* and the native bird *Coereba flaveola*. Additional objectives included to determine parameters that are responsible of the differences in reproductive success observed between trees in the wild, and to study the biology of the seeds and the seedlings.

P2355. *Consolea* (Cactaceae, Opuntioideae) an endemic West Indian genus threatened with extinction.

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Consolea (Lemaire) is a genus of flat-stemmed opuntoids endemic of the Caribbean region. *Consolea* differs from opuntoids in the continent particularly in their undetermined central stem from which asymmetrical lateral branches grow. *Consolea* is a handsome group that flowers twice a year or more. Its exotic looking and colorful flowers make them attractive as ornamentals. Unfortunately, island floras are often composed for ecologically restricted populations which are not capable of adapting to drastic environmental changes. Groups as *Consolea* inhabit coastal areas, which are particularly affected by human growth, clearing for agriculture, burning, and hurricanes. On the other hand, lack of systematic studies creates confusion on delimitation of taxa which in turns expose entire populations to be wiped out. This work presents a morpho-anatomical study supported with DNA sequences to delimitate taxa among this poorly studied group, and thus its narrow geographical distribution. We recognize nine species and three sub-species in the *Consolea* genus, and we suggest a sound management plan for their *in situ* and *ex situ* conservation.

P2356. Use of population genetic data for the *ex situ* conservation of *Crambe pritzelii* and *C. tamadabensis*, two threatened endemics from the island of Gran Canaria.

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Crambe pritzelii and *C. tamadabensis* (Brassicaceae, sect. *Dendrocrambe*) are two threatened monophyletic taxa endemic to the island of Gran Canaria that require urgent conservation action. We apply population molecular markers (allozymes) to explore the extent of genetic differentiation in 12 populations that represent the known range of occurrence of these closely related endemics and use the resulting data to (1) construe and compare their levels of genetic variation, (2) assess whether these populations exhibit significant genetic structuring in space, and (3) integrate this information to streamline sampling strategies that allow us to represent a significant amount of their genetic variation in the seed bank of the Jardín Botánico Canario "Viera y Clavijo" while minimising sampling effort and storage space. Despite their restricted distribution, these taxa exhibit levels of genetic variation that are substantially higher than those reported for other island endemics, thereby adding to the emerging picture of a higher genetic variation in Canarian plants than in those from other oceanic archipelagos.

P2357. Antheridiogen effects in the allotetraploid *Dryopteris corleyi* and its parent species

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Theoretical studies predict that increased selfing may enhance the establishment and persistence of polyploids among their diploid progenitors. *Dryopteris corleyi* is an allotetraploid fern derived from *D. aemula* and *D. oreades*. Its narrow geographic range (a 60 x 10 km strip on the North Spanish coast) overlaps that of *D. aemula*, and backcrosses, giving rise to sterile hybrids, have been detected. Isolated gametophytes of *D. corleyi* and diploid parents were cultured in a nutrient rich substratum. The gametangia sequence of the three species was protogynous. The same substratum was used as antheridiogen source in a new culture experiment. The three species were combined as source and target of pheromone action (i.e. nine species pairs). The exudates of the three species had the same intra- and interspecific effects: they slowed gametophyte growth rate and induced presexual-to-male transition. Consequently, antheridiogens favour intergametophytic fertilization and hybridization. The implications for conservation of the narrow endemic *D. corleyi* are discussed.

P2358. Rare and threatened liverworts: An overlooked area of conservation in the New Zealand botanical region.

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New Zealand has been identified as one of the twenty-five richest and most threatened biodiversity reservoirs on Earth. New Zealand's islands harbour a remarkable concentration of endemic species, including approximately 80% of its flora. Historically, New Zealand has experienced exceptional loss of habitat, and currently, invasive species pose as the largest threat to the flora and fauna. Of worldwide significance is the fact that New Zealand is home to an estimated 5-10% of the world's liverwort species, including over 200 endemic species. Moreover, New Zealand has 48 of the 73 liverwort families. First, this study identified potential threats to the New Zealand liverwort flora, and second, assigned categories of threat to develop a preliminary checklist. Selection of species was based on field studies spanning three decades, extensive herbarium records, and bibliographic references. Case studies illustrating the different categories of threat include examples from monotypic families and genera. We conclude that research relating to the conservation biology of the New Zealand liverwort flora requires great urgency and recommend areas of priority.

P2359. Floristic diversity, endemism and conservation in semi-arid Caatingas of NE Brazil

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The semi-arid Caatinga biome of Brazil covers an area of c. 1,000,000 km², occupying part of eight Northeastern States from Piauí to Bahia, and part of N. Minas Gerais. The area is climatically unstable, and severe droughts occur, yet supports a farming population who, over 400 years, have used the available natural resources in an unsustainable way. In spite of this, many well conserved areas still exist, especially on rock outcrops or shallow soils unsuitable for cultivation. Vegetation is typically deciduous woodland or scrub, with spiny Mimosoid legumes, Euphorbiaceae and Cactaceae, and during the rains, temporary pools and marshes. Caatinga supports a flora of >3000 species, with c. 10% endemism and 20 endemic genera. Biogeographic studies suggest it is part of a dry forest formation extending south into Argentina, skirting the Chaco and reaching north to the Eastern Andean Piedmont of Ecuador. Although many areas suffer serious modification by human occupation, conservation of centres of biodiversity, with appropriate water management are the only hope for long-term welfare of communities who earn their living from agriculture in the region.

P2360. The most rare and endangered vascular plants from Romania

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Romanian Red lists comprise about 1400 rare, endangered and endemic plant species. Among these we critically analyze three categories: endemic and sub-endemic species (*Ajuga salicifolia* subsp. *bassarabica*, *Centaurea triumfettii* subsp. *angelescui*, *Cochlearia borzseaana*, *Colchicum arenarium*, *Dianthus dobrogensis*, *Dianthus trifasciculatus* subsp. *parviflorus*, *Linum pallasianum* subsp. *borzeanum*, *Sausurea porcii*, etc.), relic species (*Betula nana*, *Betula humilis*, *Allium obliquum*, *Geranium cinereum*, *Nitraria shoberi*, etc.) and plants that, in our country, are at their limit of area (*Asyneuma anthericoides*, *Hedysarum grandiflorum*, *Jurinea tzar-ferdinandii*, *Genista sessilifolia* subsp. *romanica*, *Plumbago europaea*, *Salvia ringens*, *Scabiosa micrantha*, *Alkanna tinctoria*, *Thlaspi jankae*, etc.). Most of these taxa are not included in Globally or European Red Lists. In this way we hope to underline the urgent necessity to save some very rare and critically endangered plant species from Romanian flora.

P2361. Vulnerable flora of Sierra de Pachuca, Hidalgo, México: proposal of one local red list.

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The Sierra de Pachuca located in the north end of Mexico's Valley occupies a surface of approximately 600 km², there is the oldest national park of Mexico (1899): Parque Nacional El Chico.

The checklist of the Sierra de Pachuca, Hidalgo, México, was made after ten years of research, with a total of 969 species from 411 genus and 93 families, 11 gymnosperms, 755 dicots and 203 monocots.

From the total, there are 195 species of plants which geographic distribution is restricted, or their populations are of few individuals, and their permanence in this place is threatened. The set represents 20.1 % of the total flora registered by the authors on those mountains. Eleven species are strict endemics from this region, 43 are endemics from México and 141 have a wider geographic distribution, but can disappear from Sierra de Pachuca by changes in their habitats by human influence.

This region, because of its great vegetal, ecological, phisiographic and historical diversity, has a high value for the preservation of the biological diversity of the country.

P2362. The precarious future of *Lepidospartum burgessii* (gypsum broomscale, Asteraceae), a rare and vanishing taxon of the southwestern United States.

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Lepidospartum burgessii, a gypsophilic shrub, is endemic to the Alkali Flats region on the border of New Mexico (NM) and Texas. During a 1991/1992 survey, little recruitment was evident and most shrubs were mature. In 1997, our objectives were to determine *L. burgessii*'s fecundity and availability of pollinators. In 1997, about 15% of shrubs counted in 1991/1992 in NM were dead. Shrubs produced abundant flowers but no seed. Pollinator availability did not limit pollination. Acetocarmine stain was used to estimate potential pollen functionality. Over 9,500 pollen grains, confirmed to be trinucleate, were assayed. Potential functionality was low (<50%) but unlikely so low as to prohibit fertilization. Florescence microscopy was used to investigate *in vivo* pollen germination and 524 stigmas, either from open pollinated flowers or controlled crosses, were examined. Pollen either did not germinate or the tubes stopped growing prematurely on the stigma surface suggesting that a homomorphic, sporophytic incompatibility system operates. Genetic drift may have resulted in decreasing the number of S-alleles in the population so that reproduction is inhibited.

P2363. Population survey as a basis for conservation of the priority species *Centaurea horrida* Badarò (Sardinia, Italy).

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Centaurea horrida Badarò (Asteraceae), a narrow endemic species located only in northern Sardinia (Italy), is a vulnerable species according to the IUCN Red List of threatened plants, a protected species according to the Bern Convention and a priority species according to the EU Directive 43/92 "Habitat". *C. horrida* lives in rocky cliffs and internal flat areas and is fragmented into five populations. Because three of these occur in protected areas, biological and demographical studies aimed at their conservation are carried out. All the populations are mapped; the area of occupancy and the extent of occurrence are estimated. Individual census, individual size, size/age relationship by anatomical and biometrical analysis have been carried out. Data about population structure on 12 sampling areas are showed. High predominance of non-fertile achenes was recorded in all the populations. Disturbance affecting populations is related to active grazing by wild mammals in protected areas and the impact due to tourist activities.

P2364. Distribution and ecology of an endemic species, *Salicornia qassimensis* Al-Turki & Swarup ined. from Saudi Arabia.

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Salicornia qassimensis Al-Turki & Swarup is a rare species reported from Al-Waz salty area, 15 km east Qassim Province, Central Saudi Arabia. It is a halophytic, succulent, leafless looking annual plant that starts its life cycle in January-February period when the salinity is low due to intermittent rainfall. Stem usually

cylindrical, appearing composed of articulated segments. The plant continues to grow for about six months and produces several branches from the base. It starts flowering in October and November and apparently withers off in December. Only one population with fewer than 1000 plants is so far reported for this species. The population is facing severe threats from several external agencies, such as fire, competition with other plants, etc. Immediate conservation measures are required to save the population of this species.

P2365. Geographic range size and the explanation of spatial diversity patterns of New World palms

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Macroecological approaches focus on correlations between species richness and environmental predictors. Here, we investigate effects of range size in these analyses using distribution data for all native American palm species. We evaluate the contribution of range-restricted and widespread species to patterns of species richness and endemism and analyze the impact of range size on the perception of their determinants. According to their range size, species sequences were produced in ascending, descending, and random order. Sequential correlations between cumulative species richness and environmental predictors were performed. Despite the higher proportion of species with small ranges, species richness patterns are dominated by the ~20% most widespread species. Energy and water related factors account for much of the species richness patterns of widespread species but are much poorer predictors for range-restricted species. Species richness of range-restricted species is to a larger extent determined by topographical complexity.

P2366. Endemic vascular flora of Calabria (S Italy): taxonomic and conservation remarks

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The Calabria region, situated at the centre of the Mediterranean sea, is marked by 67 narrow endemic vascular plants (including species, subspecies and varieties). They belong to 25 families and 42 genera, of which the most represented are *Centaurea* (6 units); *Salix* (4); *Anthemis*, *Dianthus*, *Epipactis* and *Limonium* (3); *Achillea*, *Adenocarpus*, *Allium*, *Armeria*, *Cardamine*, *Genista*, *Hieracium*, *Salvia*, *Silene* (2); all other genera are represented by only one unit. It is noteworthy the occurrence also of one monotypic genus (*Lereschia*, Apiaceae). This endemic flora mainly grows in well defined habitats, such as beech forests, cliffs, orophilous cushion-like vegetation, primary or secondary grasslands etc. The richest areas for endemics are the Sila (13 units) and Aspromonte (9) mountains, and the dry thermo-mediterranean southernmost part of the region (7). Taxonomy, distribution, conservation status and population amount growing in protected areas for each unit was studied, also in relation to their taxonomic rank. The role and the importance of narrow endemics in the regional strategies for conservation is evaluated and discussed.

P2367. An insight into the 50 most threatened plants of China

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China harbors a very rich plant biodiversity both in number of taxa and in endemics, although a significant fraction is threatened mainly due to human activities. Between 3,000-5,000 plant species are currently endangered in China, probably falling into one of the threat IUCN categories (CR, EN or VU), although some taxa are in a situation of extreme endangerment. A few dozens of plants contain only a few individuals or they are restricted to a single locality, thus being very susceptible to both anthropogenic and natural threats. Some well-known examples are *Abies beshanzuensis*, *Pinus squamata*, *Magnolia zenii*, *Ulmus gaussenii*, *Craigia kwangsiensis*, *Gletsidia vestita*, *Carpinus putoensis*, and *Bhesa sinica*, among others. Herein we advance, in the basis of the current knowledge, a list of the probably fifty most endangered plant taxa of China, following the same philosophy of the 'Top 50' campaign launched by the Species Survival Commission of IUCN in the Mediterranean basin islands. Some basic features (distribution

area, legal status, threats, etc) are included in a brief profile for each plant.

P2368. Unique floristic complexes in Armenia

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Armenia's flora includes 123 endemic species. They can be found on the entire territory of the country, Yerevan (39) and Dareleghis (36) floristic regions, however, are the richest regions with regard to endemics. Certain small mountain ranges and volcanoes are particularly notable due to their abundance with endemic species. Examples of such territories are the area near Areni and Khachik villages in Vajk mountain range with 10 endemic species, Urts mountain range and Arteni volcano each with 9 species, mount Khustup and the southern slopes of Vardenis mountain range each with 8 species. All the mentioned territories are characterized by their aridity, diversity of soil cover and vegetation. The majority of these endemic species is neo-endemic and their abundance is arguably linked to the intensive speciation processes. Possible factors contributing to the intensity of these processes might as well be the level of aridity, proximity to geological faults, seismicity of the territory and many others.

P2369. Endemic plants of Armenia

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Flora of Armenia is remarkable by the high number of taxa confined to a small area, especially Armenian endemics. Political boundaries are artificial, but in spite of this fact Armenian endemism is of the same level as that of the naturally delimited, large Mediterranean islands, which are notoriously richer in this respect than mainland regions of a comparable size. The 123 species endemic to Armenia represent 3 % of the total Armenian flora. Most are of relatively recent origin, dating from the Quaternary or Holocene, and only a few palaeo-endemics, e.g. of *Pyrus*, take their roots in the Tertiary period. This reflects the relatively recent diversification in the region of flora, resulting in its present high diversity. The incidence of endemic taxa is found in the arid zones of the southern and central part of the country. *Centaurea sensu lato* has than 16 endemic species in Armenia, and *Pyrus* 13 whereas the largest genus, *Astragalus*, has only 6.

P2370. The Endemic Species of *Trigonella* L. and Their Conservation in Turkey

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Trigonella L. is represented with 52 taxa in Turkey, 21 of are endemic. The endemism rate is about 40.38%. Five taxa are known from type locality and they are local endemics. These are *Trigonella cassia* Boiss. (CR), *Trigonella raphanina* Boiss. (CR), *Trigonella pseuodocapitata* Contandr. & Quezel (CR), *Trigonella arenicola* Hub.-Mor. (CR), and *Trigonella cilicia* Hub.-Mor. (EN). The others are also have rare distribution and mainly localised to Mediterranean region. The distribution and threatened category of endemic plants, especially the positions of narrowly distributed endemics were not known accurately until this study. Thus, their threatened positions will be discussed with the newly observations which are obtained during the revision of genus in Turkey. In addition, the suggestions will be given for their conservation. A full photographic presentation of endemic plants and observations on their populations will be provided.

P2371. Endemics of Kazakhstan Angiospermae

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Comprehensive inventory of 160 plant families of Kazakhstan described in literature has shown 20 ones included nearly all endemics. About 25% each of Alliaceae, Apiaceae, 20% each of Boraginaceae, Lamiaceae, Liliaceae, Limoniaceae, considerable amount of Zygophyllaceae, Polygonaceae species are endemics. In general, endemic spp. are better represented in the south-east and south part of the country. Of 29 Kazakhstan floristic regions there are about 25% of endemics in each of Jungar, Zaylyshy Alatau, Karatau, about 15% in the west Tian-Shan and Balkhash-Alakol (including instances of "double counting"). The relative

distribution of each family endemics varies from region to region. For example in Karatau, Jungar Alatau there are considerable number of Apiaceae, Lamiaceae endemics. Alliaceae and Liliaceae endemics are in Zaylyisky Alatau and Chu-Ilyisky mountains. In Balkhash-Alakol there are numbers of Zygophylaceae, Lamiaceae ones. The considerable amount of Polygonaceae endemics is in Zaysan, Balkhash-Alakol, Kzyl-Kum desert. For all that the most of endemics grow in dry, hot environments, in mountains on stony, road-metal slopes and rocks.

P2372. Taxonomic Consideration of Glandular Trichome Types in Greek Taxa of the Genus *Thymus* (Lamiaceae)

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The genus *Thymus* L. (Lamiaceae) is a polymorphic and taxonomically difficult group of c. 350 taxa. In Greece, c. 35 species and subspecies have been reported, a few of them endemic and very rare. In spite of its interest a limited number of micromorphological studies can be found in literature.

Micromorphology and anatomy of glandular trichomes on vegetative and reproductive organs of 18 representative *Thymus* taxa (43 samples) have been studied using light, fluorescence and scanning electron microscopy. Observations were mostly made on dry material collected in Greece. The comparative use of fresh material in several taxa did not reveal any noteworthy differences. Altogether, thirteen glandular trichome types have been found; eleven of them are reported for the first time in the genus *Thymus*; two of them are new records for the Lamiaceae family. Organ traits such as glandular trichome types, their density and distribution appear to be of taxonomic value. The observed micromorphological stability among members of the same section is of economic importance, as it offers a sound ground for the exploitation of secondary metabolites.

P2373. Introduction and selection of non-traditional horticultural plants in the National Botanical Gardens of Ukraine

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The National Botanical Gardens of Ukraine was founded in the 1935 year. The main direction of the researches there are reservation of biodiversity and introduction of different varieties of plants of Ukrainian natural flora, medicinal, horticultural, ornamental plants.

The traditions of introduction and selection of the non-traditional plants were began in the Kyiv by academician M. Kacshenko in the began of XX c. Rich genetic pool of non-traditional horticultural plants has been accumulated. Many such varieties of plant were realized thanks to high food and medical properties of their fruits. The perspective sorts of *Actinidia arguta*, *A. colomicta*, *A. polygama*, *A. purpurea*, *Cornus mas*, *Cydonia oblonga*, *Chaenomeles japonica*, *Diospyros kaki*, *D. virginiana*, *D. lotus*, *Hippophae rhamnoides*, *Eleagnus multiflora*, *Lonicera edulis*, *Mespilus germanica*, *Morus alba*, *Sambucus nigra*, *S. canadensis*, *Sorbus aucuparia*, *S. domestica*, *Shepherdia argentea*, *Viburnum opulus*, *Ziziphus jujuba* have been incorporated in "The Register of Plants varieties of Ukraine".

P2374. Landscaping and living collection on the Extreme North-East of Eurasian continent

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Magadan is 100 000 people town on North-East Russia (59 30' N 151 E) on sea of Okhotsk founded in 1939. It is located in zone of "islandic" permafrost. Peculiarity of severe climate is late spring: leaves appear on trees early June or even middle June. The first small arboretum on area 0.17 ha was established in 1935 before Magadan was awarded town status. 53 species of trees, shrubs and herbs were registered in it in 1993. They are mostly native species except *Malus* sp., *Caragana frutex*, *Syringa josikaea* and

Convallaria majalis brought as seedlings, seeds and rhizome in 1949 from St-Petersburg. They bloom but no fruits. Parks in Magadan contain native species. On the streets also native species of trees and shrubs planted except *Padus maackii* on Gagarin street and *Pinus sylvestris*, *Picea obovata*, *P. ajanensis* and *Malus* sp. in vicinity Magadan. In 2000 experiment in Nagaevo Bay on introduction of species of Magadan region and adjacent territories of the Russian Far East was started. Winter 2002-2003 was unusual - snow was absent till 7 January and $t = -26.1^{\circ}\text{C}$. Nevertheless not only many native species but also some plants from Ussurian region, China and Kamchatka overwintered.

P2375. The species, form and sort variety of the genus *Corylus* L. in Ukraine

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The data about the collection of species and forms of the genus *Corylus* L. growing in Ukraine and the overview of the history of foundation, restoration and conservation of the National dendrological park "Sofiyivka" is given in the abstract. Nowadays Ukraine is on its first step in growing *Corylus* L. National dendrological park "Sofiyivka" is the the only place in Ukraine where a great collection of the genus *Corylus* L. was collected. One of the species *Corylus avellana* L. is native for Ukraine the other species were introduced from their native growing places. The data about the wide collection of the genus *Corylus* L. in the National dendrological park "Sofiyivka" is given. Species, forms, sorts and hybrids of *Corylus* L. growing in the National dendrological park "Sofiyivka" are the valuable base for filbert breeding. National dendrological park "Sofiyivka" is a masterpiece of landscaping architecture and a scientific institution at the same time.

P2376. Complex Education for Biodiversity Conservation in Botanic Garden of Tver State University: Towards Regional Perspective.

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In the presentation the regional biodiversity conservation work done by Botanic Garden of Tver State University will be described. Since 1999 onwards Botanic Garden of Tver State University has started complex project "Biodiversity Conservation Strategy of Tver Region" which is meant to facilitate biodiversity conservation in the region. Alongside with endangered plants special attention is paid to the following aspects: specific characteristics of ecosystems, harmonious interaction of the man and nature, the art of development recreational zones and landscape design in urban environment. Scientific research carried out in the Garden provides necessary content and methodological basis for educational activity focused on regional biodiversity conservation problems. In the framework of the project great attention is paid to the raising of biodiversity awareness of the population of Tver Region. Garden employs a wide range of awareness activities: plant expositions "Living red data book", guided tours, lectures, interactive games, joint pilot-projects with NGO, mass media projects, publishing activities, family holidays, festivals etc.

P2377. Aburi Botanic Gardens Role in Conservation of Medicinal Plants in Ghana

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The World Health Organisation estimates that up to thirty percent of the World's population rely on plants for their primary health needs. In Ghana most people in rural areas depend almost entirely on herbal medicine. Even in urban areas where orthodox medicine is available there is considerable reversion to herbal cures due to the high cost of conventional medicine. All plants used in primary health care in Ghana are collected from the wild by over 500,000 practitioners. These herbs are thus threatened not only by habitat destruction but also by the unsustainable harvest from the wild.

The need to initiate actions to reduce the pressure on these treasures became the concern of Aburi and of other stakeholders. Based on results of ethnobotanical surveys undertaken by Aburi, with interviews in selected communities, a concept was developed and activities initiated to conserve threatened and most frequently used plants. The on-going efforts to promote sustainable use of local plants for local needs and thus to improve the livelihood of

local people is Aburi's contribution to conserve medicinal plants for the present and future generation.

P2378. The greenhouse collection of succulents in Saint-Petersburg's botanical garden during the World war 2 and the reconstruction of the collection after the war

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St. Petersburg's Botanical Garden was founded by Peter the Great in 1714.

World War 2 brought a lot of harm to all living collections of the Botanical Garden. In the year 1941 over 50 inflammatory bombs were thrown down on the territory of Garden; and in 1942-1943 85 artillery missiles more, as a result practically all the greenhouses were destroyed. During the Leningrad siege nearly all the tropical plants died, and the biggest part of the saved species were cacti and other succulents. Gardeners kept them in their own flats. According to official data from 1947 10 samples of succulents, representing 19 families, 75 genera and 354 species had survived the wartime.

In 1946 big number of plants arrived from the Sukhumi dendrarium-park, including agaves, yuccas and others. The other resource of restoring the collection was the delivery of plants from the defeated Germany following to reparations in 1945 - 1947. In 1949 a lot of succulents were bought from the German firm "Kakteen-Haage".

Nowadays the succulent collection consist of 2302 taxa (305 genera, 35 families) and overcomes the collection of the Emperor's Botanical Garden in its best times by number and contents.

P2379. Conservation and Utilization of Plant Genetic Resources in Nepal

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The plant biodiversity of Nepal consists: cereals, grain legumes, vegetables, industrial crops, oilseeds, spices, fruits, ornamentals and herbs. The contribution of plant biodiversity to food supply, agriculture, forestry, medicine and industry is enormous. Nearly 54 percent of Nepal's physical landscape is covered with vegetation (37 % forested area, 5 % shrub land and 12 % grass land). A total of 118 ecosystems with 75 vegetation and 35 forest types have been identified in Nepal. Though Nepal possesses only 0.1% of the total landmass of the world, it harbors nearly 2.5 % flowering plants, 3% pteridophytes and 6 % bryophytes of the world's flora. There is high degree of endemism (246 species), which accounts for 30 % for whole of the Himalayan region. There are more than 6,500 plant species of which more than 500 plant species are edible, 700 species of medicinal plants have been identified and a total of 10,736 accessions of 90 crops have been documented. Nepal is considered to be one of the countries of origin of rice in the South Asia. Existence of more than 120 species of wild relatives of cultivated crops is an additional dimension to the plant genetic resources of Nepal.

P2380. How selfing and intra- and interspecific crossing influence seed set, morphology and ploidy level in *Euphrasia*: An experimental study of species occurring in the Alps of Switzerland

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Annual alpine species rely on selfing rather than on cross-pollination for successful reproduction. However, insect visits may occasionally cause cross-pollination not only within but also between closely related species. The aim of this study was to investigate four species of *Euphrasia* for their efficiency in spontaneous selfing and their success in intra- and interspecific crossing. We used the seed sets that followed spontaneous selfing and artificial cross- and self-pollination to measure the breeding success. We verified the hybridogenous origin of plants resulting from interspecific crosses using RAPD patterns and determined their ploidy level using flow cytometry. While spontaneous seed set was high in the two small-flowered species, seed set in the large-flowered species was small and affected by external circumstances. F1 and F2 hybrids from interspecific crosses of two diploid species were obtained, including polyploid individuals in both generations.

P2381. Species relationships within *Arisaema* (Araceae) as inferred from an improved chloroplast phylogeny

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Arisaema comprises 150 species of herbs that occur from the Himalayas to Japan and SE Asia; three species occur in the New World, a few in East Africa, and one ranges from Africa across Arabia to Nepal. Our earlier analysis (Am. J. Bot. 91; 881-888, 2004) of chloroplast sequences from representative species of ten of the eleven sections recognized by Murata (1984, 1990, 1991) showed that several sections are polyphyletic. We have now added data from the psbB-psbH cpDNA intergenic spacer as well as numerous additional species, especially from Japan. Combined rpl20-rps12 and psbB-psbH data reveal that *A. flavum*, the above-mentioned widespread species and only member of section Dochafa, is sister to the Asian *A. jacquemontii*, and that both are sister to *A. tortuosum* and related Asian species from section Tortuosa Engl. The remainder of section Tortuosa, including the American *A. dracontium* and Mexican *A. macrospatum*, is confirmed as highly polyphyletic, implying that the striking spadix appendages to which the section owes its name evolved several times in the genus.

P2382. Prospects of biofuel production from plants in India

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Plants undertake photosynthesis to convert light energy to chemical energy

and some plants convert it to directly to liquid fuels. Laticiferous plants yield hydrocarbons which provide liquid fuels. The methods for obtaining liquid fuel from plants varies from plant to plant and the contents vary from season to season. Different factors promote and regulate the biosynthesis of hydrocarbons in plants. Detailed investigations have been carried out on the selection of plant species providing maximum hydrocarbon contents. The optimal conditions for the maximum latex biosynthesis have been determined. Different inorganic fertilizers have increased the latex biosynthesis and phosphorous is one of the most important factor to promote the hydrocarbon yield. Plants could be used to provide renewable and clean biofuels. India has over 130 million ha of wasteland which could be utilized for biofuel production. Details shall be presented

P2383. Is competition important in alpine plant communities? Removal experiment in the Northwest Caucasus

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The aim of our study was to answer two questions: 1) is the net effect of the surrounding plant community to stress or benefit different alpine species in comparison with growth alone? 2) How does the role of competition change among communities with different productivity? We use a method of removing all but one species from plots within a community to create the monocultures. Role of competition as community structuring mechanism was estimated according to Goldberg (1994) approach. The experiment was conducted during 6 years in alpine area of the Teberda State Reserve, Russia. The importance of competition in the communities increases in the order: *Festuca varia* grasslands < alpine heaths, snowbeds < *Geranium* - *Hedysarum* meadows. Competition was more important in the most productive alpine community. Unexpected intermediate position for low-productivity communities of stressful environment and the last position of relatively productive *Festuca varia* grassland were obtained. Our results support Fretwell-Oksanen hypothesis that competition in communities of intermediate productivity is less important than in low or high productive communities.

P2384. Preonanthus: An Independent Genus Formed by Alpine Ranunculaceae Taxa

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Pulsatilla, genus of Ranunculaceae family, contains more than 170 taxa, but the objective correlations between these categories are still unclear. The morphologically variable species make the

correct classification really difficult. However, there are some taxa (*P. alpina* and its subspecies, variants), which show significant morphological difference from other *Pulsatilla* species. We tried to support this difference with phylogenetic studies.

Molecular phylogeny was constructed using nucleotide sequences of internal transcribed spacer (ITS) regions of nr DNA. A phylogenetic tree was constructed from the nucleotide sequence alignment using PAUP* 4.0. ITS regions seem to be valuable source in identifying different species, while significant polymorphism manifested in most of the studied taxa at species level. 2 samples showed significant polymorphism at generic level. While these species are exactly the same, which differ in numerous morphological characters from all the other *Pulsatilla* taxa and were classified as *Preonanthus* species, we think these taxa should be considered generically different and *Preonanthus* genus should be recalled.

P2385. Resources of Harboi Rangeland Kalat, Pakistan as affected by Afghan Refugees

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Harboi rangeland, Kalat is degraded juniper forest with arid temperate climate. The study conducted during 1999-2001 revealed that there were 202 species of 45 families. The life form and leaf size spectra of flora exhibited xenomorphic features. *Juniperus polycarpus* was the only tree species. The plant communities were classified into *Bromus* type, *Seriphidium* steppe and *Juniperus* types during spring while it shifted to *Seriphidium* steppe, *Astragalus* steppe and *Juniperus* types during summer. Species diversity during summer (0.16-0.07) was high than in the spring (0.12-0.05). Species richness ranged from 1.67 to 2.6 in spring and 1.1 to 3.41 in summer. The over all vegetation is highly degraded and open with scattered individuals of juniper. The rangeland is suffering from overgrazing, deforestation and erosion. The degraded range condition needs ecological restoration for the sustainable future use.

P2386. Impact of Afghan Refugees on vegetation along Durand Line, Pakistan

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The Pak-Afghan border stretches from 1400 to 5000 m height with many small and large passes. Traditionally people along borderline freely cross the borders. During 1978 more than 3 million afghan refugees fled into Pakistan that affected the vegetation and plant diversity. The southern borders have *Artemisia-Astragalus* scrub. At Mohammand and Arundu, *Olea-Reptonia* forests changed to scattered trees. *Quercus baloot* and mixed blue pine forests occur at Dir. The alpine borders of Chitral have *Artemisia*, *Acantholimon-Acanthophyllum* steppe, alpine pastures consisting of low, open, non-palatable plants. The coniferous forests along Parachinar border have been degraded. At Bajaur border oak forests have shifted to *Dodonaea* scrub. Overgrazing and deforestation has decreased the over all productivity of forest and rangeland resources and increased soil erosion. There is need to restore these resources for sustainable resource management.

P2387. Testing the geographic transposability of predictive distributions models of plant species under present and future climate scenarios

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Predictive habitat distribution models have been increasingly used to assess the impact of climate change on plant species. In this context, models applicable to a wide range of geographic and environmental situations are highly desirable. Models based on niche theory fit a relationship between field observations and environmental predictors, and project it into the geographical space. Due to their empirical nature, models rather fit the realized than the fundamental niche of species. For this reason, it has been

argued that these models have their application limited to the specific regional and temporal domain of calibration. In order to verify this, we reciprocally fitted distribution models for more than 100 plant species in two regions of the European Alps: the western Prealps (Switzerland) and the Northern Calcareous Alps (Austria). This study aims at answering the following questions:

- 1) Do environmental response models of the same species differ between the regions?
- 2) Can we predict plant species distributions of one region using models of the other region and under present and future climate conditions?
- 3) Do future scenarios differ across regions?

P2388. Post-agricultural succession in areas of the Atlantic Forest, Brazil.

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Clearing forest for agriculture is one of the most common disturbances in tropical regions. In order to understand post-agricultural recovery and promote management projects, studies on succession need to be conducted. Studies of post-agricultural recovery may have been neglected because of the assumption that disturbances have the same effect. Post-agricultural areas are likely to be different from treefall gaps because they are larger in area and have different history, and, consequently, produce more impact on the environment. This study describes the process of succession in post-agricultural areas, within Mata Atlântica domain. A total of 0.5ha was established in each area. A survey about the dominant species was performed in order to estimate the percentage of Poaceae and Pteridophyta. All trees taller than 1.80m (the average height of *Pteridium aquilinum* stands) were tagged and measured. The most common families were Euphorbiaceae, Labiateae, Apocynaceae, Myrtaceae, Simarubaceae, Erythroxilaceae Chrysobalanaceae, Lauraceae, Leguminosae, Rutaceae. Financial support from CNPq/ASCIn.

P2389. Evaluation of the ozone-related risk for Austrian forests

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Forest ecosystems are particularly affected by the impact of stress factors. Apart from natural stressors, there are also anthropogenic ones such as air pollutants. Ozone is considered to be the most phytotoxic air pollutant in Austria on a regional scale. In the past decade, concentrations in forested areas have increased significantly by up to 1.6 ppb per year. In order to assess the ozone impact on forests, a nationwide mapping of the AOT 40 for the forested area of Austria was done with the objective to take the ozone Critical Level as defined by the UN-ECE as the basic concept and to develop a threshold value with the help of three approaches. The adaptation of trees to the pre-industrial ozone levels, the parameters affecting stomatal uptake such as light intensity and water vapour saturation deficit, and the hemeroby (altered and natural) of the forest stands were taken into consideration. 61 % of the forest area showed a level of more than 10,000 ppb.h. Lower altitude areas with predominantly altered stands were affected more heavily.

P2390. Diversity of vascular epiphytes on isolated remnant trees in the Andes of southern Ecuador

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We studied the diversity of vascular epiphytes on isolated remnant trees of pastures at Estación Científica San Francisco (ECSF) in the lower montane forest belt of southern Ecuador (1800-2200m a.s.l.). The objective of this study was to document the importance of remnant trees for the survival of epiphytes following forest clearance. Twenty-one canopy trees (15 remnant trees, 6 forest trees) were divided into five zones following Johansson (1974). Bromeliaceae, Orchidaceae, Piperaceae and Polypodiaceae were relatively well represented on remnant trees in terms of species richness and abundance. Other families commonly found on forest trees such as Dryopteridaceae, Ericaceae, Grammitidaceae and Hymenophyllaceae were poorly represented on remnant trees.

Diversity, species richness and abundance of epiphytes were significantly lower on remnant trees as compared to forest trees. Impoverishment was greatest on the stem base and in the outer crown, and least in the inner crown. We suggest that microclimatic changes and increased drought stress following the isolation of the remnant trees resulted in lowered rates of establishment and survival of epiphytes.

P2391. Historical land-use legacy and *Cortaderia selloana* invasion in the Mediterranean region

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Land-use changes and species introductions threaten native species conservation. Mediterranean areas are highly susceptible to invasions and they have suffered major land-use changes, yet there is scarce research to link biological invasions and land-use change attributes: number, type, trajectory and direction. Distribution and abundance of *Cortaderia selloana*, an alien species, was analysed in 332 fields in Empordà marshes (NE Spain). We related invasion patterns to land-use spatiotemporal data (1956-2003) to determine land-use susceptibility to invasion in the last 5 years and to find out which attributes triggered invasion. Invaded fields (22.30 %) are near to urban areas and its density has been triplicated since 1998. Invasion was over-represented in pastures and old-fields and it increased with time since abandonment. *C. selloana* presence was associated to land-use changes, the most invaded fields were the ones that remained as undisturbed. Plant biomass was higher in agricultural field margins and in fields that had a disturbed land-use in 1956 and 2003. Overall, *C. selloana* invasion pattern can be explained by the historical land-use legacy.

P2392. The Northern border of the area of *Pteridium aquilinum* L. in the West-Siberian plain.

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Pteridium aquilinum is a cosmopolitan species. Its habitat has been studied in many countries (Page, 1976, 1997). But in West Siberia its distribution is not quite clear and the northern border of the area is interpreted in different ways. P.N. Krilov (1927) and L.I. Krasnoborov (1988) pointed out the territory near the town Tobolsk (1° 58'N), R.M. Tryon (1941) that of the town Beresovo (1° 64'N), A.I. Shmakov (1999) that of the Ob's geobotanical district (1° 67'N). We investigated the geographical distribution and coenotic value of *P. aquilinum* in 2001-2004. The research area included forest-steppe and forest zones of West Siberia near the villages Sladkovo, Demianskoe, Salim, touns Ishim, Tuemen, Tobolsk, Langepas, Nizhnevartovsk, Surgut, Hanti-Mansiisk, Sovietsk, and in Ugansk and Malososvinsk nature reserves.

As a result of our analysis, the Northern border of *P. aquilinum* is determined. It passes near Arantur lake in the national park «Kondinskii lakes» (1° 60,5'N) (Red data book HMAD, 2003 and our data). So it is more to the south of Tryon's and Shmakov's directions but to the north of those by Krilov and Krasnoborov.

P2393. Land use changes in savannas of Central Brazil: The impact of eucalypt plantations and *Brachiaria* pastures on water fluxes

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We compared transpiration in natural savanna, *Eucalyptus grandis* plantations of two different ages, and grazed and ungrazed pastures of *Brachiaria decumbens*. Water extraction in the *Brachiaria* pastures was mostly restricted to the superficial soil layers whereas *E. grandis* plantations and savanna vegetation accessed deeper soil layers. There was a strong linear relationship between basal area and active xylem area for both *E. grandis* and savanna trees. Stem cross-sectional area was mostly conducting tissue in *E. grandis*, but only about 36% in savanna trees. Thus, over a range of 20 to 200 cm² of basal area, *E. grandis* maintained daily sap flow rates that were 2 to 3-fold higher than savanna trees

in the wet season. Sap flow rates of *E. grandis* increased in the dry season while savanna trees maintained rates similar to the wet period. Water loss in the ungrazed *Brachiaria* pasture was higher than that of the savanna groundlayer in the wet season. Water loss in both decreased substantially in the dry season. Thus, these changes in land use have an important impact on the hydrological cycle. CNPq, FINATEC, NSF.

P2394. Regional impact assessments of multiple environmental changes for plant diversity

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Climate changes, the withdrawal from traditional land use practices, and invasion of alien plant species expose biodiversity to unprecedented stresses. The assessment of these impacts is challenging, particularly its interacting effects. Environmental envelop models in combination with comprehensive spatial data on environmental condition provide an opportunity to assess the consequences of multiple pressures for biodiversity. Here we present the results of several regional modelling studies by emphasising feasibility, potentialities and pitfalls of the models being applied. Scenarios of the impact of climate change and the abandonment of traditional pastures in alpine areas were developed for a region in the European Alps. Another impact assessment was carried out for a Chilean island where alien species pose severe threat to the endemic flora. The results of these studies showed that ecosystems are differentially vulnerable to environmental changes, that it is essential to consider multiple pressures, and that the models being applied can answer only part of what would be necessary to define effective measures for biodiversity conservation.

P2395. Primary successions in the new Aral seashore

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During first years succession is controlled according to models of facilitation and neutrality. A model of tolerance has started after lowering of ground water table and organic matter discharge of bottom deposits.

Psammosere

Initial plant colonization has started by aggregation of *Suaeda acuminata* and *Atriplex pratovii*.

Steady desalinization of light lithology soils occurs in 10 years of continental regime. The model of facilitation has become the main motive force of succession again. Transition towards tree-shrub stage with sub-shrubs (*Haloxylon aphyllum*, *Calligonum* spp., *Artemisia arenaria*) follows after 35-40 years of succession. Indexes of plant species diversity show low stability of species richness, high coefficient of community and a positive trend of evenness index.

Halosere

Soils are of heavy lithology. Desalinization of top horizons has seasonal character. Succession is controlled by a model of tolerance. Changes of dominants are as follows: *Salicornia europaea* (1-4 years) *Climacoptera aralensis* (5-8) *Halostachys caspica* (12-17) *Halocnemum strobilaceum* (20). Indexes of plant species diversity have a tendency towards decreasing.

P2396. Deforestation effects on soil physical and chemical properties in a western Kenyan rainforest, Kakamega forest

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Deforestation has significant effects on biogeochemical and physical properties of soils, which in turn influence global nutrient cycling. In this study, changes of soil physico-chemical properties caused by converting primary forest to shrubland and grassland in Kakamega tropical rainforest up to 350cm depth were evaluated. Generally, the concentrations of nutrient cations, total C and N, and EC decreased with depth except in the grasslands whereby total K and total Mg increased with depth. The forest soils had significantly higher concentrations of available K, available Ca, available Mg, pH and EC than in shrubland and grassland ($p < 0.05$) at all depths. Total C, total N and available Mn did not vary

significantly between the vegetation types. The forest soils had higher soil stocks of C, N, Ca, and Mn than the grassland and shrubland soils but lower stocks of K and Mg. It can be concluded that, the forest cover provides the most effective means for regeneration of Kakamega soils.

P2397. Modelling of forest growth in the Centre of European Russia at different land-use

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The system of models, EFIMOD was used for the simulation of ecosystem dynamic under the different forest management scenarios. The system consists of tree sub model, soil sub model (ROMUL) and statistical climate generator (SCLISS).

The forest selected for the simulation modeling is situated on Central East European Plain with continental climate of the border between coniferous and broad-leaved forest and consists of 104 forest inventory units.

Four simulation scenarios were compiled for the analyzing of forest dynamic at different silvicultural regimes: a) natural development; b) Russian authorized practice; c) selective cutting system; d) unauthorized practice.

The EFIMOD runs have been performed for 200-years stand growth and soil changes in every forest compartment for four scenarios of forest management. The results obtained allow for the conclusion on the advantage and shortcomings of different regimes of forest management for their correspondence to the concept of sustainable forest management. Selective forest technology has clear advance over all other regimes with cuttings.

P2398. Statistics of Change for Land Cover In Mexico

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Through the years the land cover in Mexico has seen big changes in its original distribution and its floristic composition sometimes drastically.

As a reference the experience acquired during 35 years of geographic activity at INEGI and specifically in elaborating the information of land use and land cover 1:250 000 scale. The statistics of change in land cover in Mexico throughout 30 years are presented in this work.

The information for setting up statistics are the vectorial files of the land use and land cover maps elaborated during three periods of time or time series: Series I (80's), Series II (90's) and Series III (2002).

All this information provides surface data of land cover (natural and induced vegetation at a level of vegetation type and secondary stages as arboreal, shrubs and herbaceous.) and land use (agricultural and crops). The objective of presenting in a clear way the changes in the types of natural and induced vegetation.

For each series is possible show the country percentages by areas without original vegetation:

80's: **20.66 %**

90's: **21.28 %**

2002: **25.97 %**

P2399. Post-fire resprouting in woody plant species of a Brazilian savanna.

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The objective of this work was to evaluate resprouting patterns after annual fires in woody plants, in an open savanna phytophysiology, at the Ecological Reserve of Brazilian Institute of Geography and Statistics, Brasília-DF, Brazil. The area was protected against fire for 23 years and it was burned in 1998, 1999 and 2000. All plants of woody layer with diameter larger than 2,0 cm at 30 cm from soil level were marked and identified. After prescribed fires, damage type (total or partial) was determined. The number of basal and underground sprouts was measured after prescribed fires. The diameter and height were measured for each marked sprout. A total of 1307 sprouts were measured. The main impact of fire on resprouting was the decreasing of sprouts number, with 684 sprouts after the first burning to 248 sprouts after the third burning. The frequent fires did not affect the sprouts size. However, it was observed a high mortality rates of sprouts, after

the second and third burnings (34,8% and 37,8%, respectively). The results showed that the interval between fires should be insufficient for sprouting regeneration of woody plants in the current Cerrado fire regime.

P2400. Conservation of biological diversity in Vyatsko-Kamskii region on a base of ecological optimization of structure and functions of agriculture landscapes

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Forming of agriculture landscapes in the Udmurt republic (UR) (a part of Vyatsko-Kamskii, VK region) has been started since the beginnings of agriculture and live-stock farming there (about 2500-3000 years ago). The result is a sizeable transformation of landscapes. We have investigated plant cover conditions in UR, rare plants and vegetation communities. We have started a project of the ecological optimization of structure and functions of the agriculture landscapes in VK region on a base of ecotopes ecological conditions and social concerns of the natural and anthropogenic lands taking into consideration the necessity of biodiversity conservation at all landscape levels. We have shown a necessity of the expansion of the nature-conservative measures from the highly protected natural territories to the whole regional level. Highly protected natural areas occupy no more than 3 per cent of the republic square and there are amassed no more than 30 per cent of the UR species diversity.

P2401. The modern status of flood plain ecosystems in the broad-leaved forests zone (river basin middle Elbe and Sejm) in connection with changes of river flow and climate conditions

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Regulated of SHC (small and middle low-dammed hydrotechnical construction) lead to stabilization of humidifying regime and to change of fluctuations GWT (ground water table) in floodplain that produces change of all components of ecosystems, change of soil and vegetative covers.

At the end of the XX century a trend of decreasing the fluctuation amplitude of water flow and level is observed in the middle Elbe and Sejm rivers basins. This is a serious cause for increasing the groundwater depth in the vegetation period, for the gley formation in upper and middle soil horizons and hence for injuring the natural wood-shrubby vegetation (*Quercus robur*, *Ulmus* spp., *Tilia* spp., *Salix* spp. etc.) in the river floodplain.

From the end of 70s in 20th century the periodically changing frequency of floods has being destroyed and leads to degradation of forest communities (*Quercus* and *Ulmus*) on the upper flood plain terraces.

P2402. Change of main type of flora of the south of the Aral Sea region as a result of environment degradation

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Modern vegetation covering of the Aral Sea basin consists of tugai, psammophytic and halophytic types of plants. Flora of this region has 176 endemic species, 15 relict species, and 33 wild congeners of the culture plants. Families Chenopodiaceae, Asteraceae, Polygonaceae, Bogarinaceae, Cyperaceae, Crophyllaceae and Tamaricaceae have most diversity of species.

Habitat's environment changes have various consequences for many types of plants in. There are deepest changes of flora in the deltaic plains of the lower course of Amu-Darya. Portion of the flooded holmme decreased from 35% to 5%. Part of the hydromorphic plant communities thinned from 40% to 25%. In the same time number of plant association, representing salted and desertified sites, increased from 25 to 70% of total area of delta.

Psammophytic vegetation (*Ammodendron*, *Salsola Calligonum*, *Artemisia*, *Carex* etc) could be described as rarefied low-productive communities with predominance of non-pabular plants. Halophytic vegetation (*Salsola*, *Halostachys*, *Karelinia*, *Zygophilum*) increases its area. Special measures should be developed to maintain natural plant resources of the Aral Sea region.

P2403. Evolution of Neogene climate dynamics - forcing and results**H. Freund;**

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The Cenozoic was an important period in Earth's climatic history, since it records the change from greenhouse climates during the early Palaeogene to icehouse climates in the Quaternary. Some of the causes of the centennial to millennial scale climate fluctuations during the Quaternary are clear but others are not well understood up to now. The main forcing mechanisms have been considered as changes in the Earth's orbit that result in periodic variations of solar radiation, the tectonic development of the earth and solar variability. These external mechanisms influencing climate changes forced internal feedbacks within the climate system e. g. changes within the internal ocean or atmospheric circulation dynamics. There is a need for interdisciplinary palaeoecological and palaeobotanical investigations to understand the importance of these complex causal factors in the initiation, evolution, and variability of the quaternary climatic system. Furthermore it is important to understand the mechanisms of "natural climatic variations" to compare them with climate changes forced by man which play an important role in today's climate discussions.

P2404. Lateglacial vegetation and climate changes in the high mountains of Bulgaria (Southeast Europe)**E. D. Bozilova, S. B. Tonkov;**
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The Lateglacial vegetation history in the high mountains of Southern Bulgaria (Rila, Pirin, Western Rhodopes) is reconstructed by means of pollen analysis, plant macrofossils and radiocarbon dating of sediments from lakes and peat-bogs located between 1300 and 2200 m a.s.l. The vegetation response to the climate fluctuations after 13000 ¹⁴C yrs. BP in the Rila Mountains is bound for the first time to a detailed chronological framework. Two stadial and one interstadial phases are delimited analogous with the Oldest Dryas-Bolling/Allerod-Younger Dryas cycle for Western Europe. During the stadials mountain-steppe vegetation composed of *Artemisia*, *Chenopodiaceae*, *Poaceae* and other cold-resistant herbs dominated at high elevation with sparse stands of *Pinus*, *Betula*, and shrubland of *Juniperus* and *Ephedra*. The climate improvement in the interstadial resulted in the initial spread of deciduous and coniferous trees (*Quercus*, *Tilia*, *Corylus*, *Carpinus*, *Abies*, *Picea*) from their local refugia below 1000 m. The palaeoecological record from the climate deterioration during the Younger Dryas is documented in thin sections of the cores investigated.

P2405. Late Tertiary/Early Quaternary range splits of the Eurasian steppe plant *Clausia aprica* (Brassicaceae)**A. Franzke^{1,2}, H. Hurka^{1,2}, D. Janssen^{1,2}, B. Neuffer^{1,2}, N. Friesen^{1,3}, M. Markov^{4,5}, K. Mummenhoff^{1,2};**¹University of Osnabrück, Osnabrück, Germany, ²Department of Biology, Systematic Botany, Osnabrück, Germany, ³Department of Biology, Botanical Garden, Osnabrück, Germany, ⁴State University of Tver, Tver, Russian Federation, ⁵Department of Geography, Tver, Russian Federation.

Several vegetation belts stretch continuously from Europe to Asia, taiga and steppe being most prominent. Numerous plant species within these vegetation belts share a conspicuous distribution area, which is longitudinally contracted or disrupted approximately along longitude 70°E. To date no hypothesis for this intriguing distribution pattern has been put forward. We detected molecular footprints in the contemporary genetic composition in nuclear DNA (ITS1, ITS2) and chloroplast DNA (*trnL-trnF* spacer region) of the steppe element *Clausia aprica* (Brassicaceae) providing evidence for a severe longitudinal range split and genetic differentiation east of the Ural Mountains about 1 million years ago caused by Quaternary climatic oscillations. *Clausia aprica* provides the first phylogeographical analysis on the intraspecific evolution of an Eurasian steppe plant.

P2406. Late Pleistocene vegetation history at Colli Euganei (Padova, Italy)**A. Miola¹, S. Piovan², G. Valentini¹;**¹University of Padua - Dept. of Biology, Padua, Italy, ²University of Padua - Dept. of Geography, Padua, Italy.

The Colli Euganei is a hill area in the Venetian alluvial plain. It has been considered a glacial refugium for thermophilous plants during LGM and actually some Mediterranean elements (e.g. *Cistus salvifolius* L., *Erica arborea* L., *Arbutus unedo* L., *Spartium junceum* L.) survive in the area. The studied site is located in a perieuganean depression (3 m a.s.l.) in the south-eastern part of Colli Euganei near the village of Galzignano. The site location is interesting because it is related both to the surrounding hills and the alluvial eastward plain (5-7 a.s.l.). Pollen analysis of a 50 m deep core reveals the main features of the vegetation history from the Last Interglacial to the Last Glacial Maximum (MIS 5, 4, 3?, 2). The chronology of the sequence is based on three radiocarbon dates in the upper part of the core. Pollen data have been correlated with 150-ky core records from the south-eastern Po Plain. It is noteworthy the presence of *Abies* cf. *nordmanniana*, *Keteleeria* and *Zelkova* at the bottom of the core.

P2407. Dendrochronological evaluation of historic changes in Lake Stirniai (Lithuania) water level**I. Stasytyte, R. Pakalnis;**

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Dendrochronological research was carried out on *Pinus sylvestris* L. timber extracted from Lake Stirniai (55°15'04" latitude (N) and 25°38'49" longitude (E)). As a result, the tree ring width floating chronology spanning 213 years was compiled. Radiocarbon dating of the samples indicated that pines grew from 1103±80AD to 1315±80AD.

Fragments of stems and roots of pines were found waterlogged in 1 m water layer. It means that in the Medieval Warm Period, the ground water level in the habitat of archaeological pines was below the nowadays lake level for at least by 1 m.

The growth conditions became unfavourable in ~1270±80AD. At the end of the 13th century the climate became damper, lake transgression started, the trees of Scots pine became dry. The basic cause of transgression seems to be the bogged bed of the rivulet Stirna flowing out of the lake. This phenomenon was caused by the changed climate conditions.

The formant analysis of the radial increase of the stems of archaeological Scots pine in Lake Stirniai revealed 52,9; 30,3; 21,1; 17,5; 15,0; 11,2 year long-term fluctuation cycles. Comparison of the obtained data with the present cycles is foreseen.

P2408. Human impact on the vegetation of Upper Dnister Plain (Western Ukraine) during the Holocene**N. Kalinovykh¹, K. Harmata²;**¹Division of Botany of National University, Lviv, Ukraine, ²Institute of Botany of Jagellonian University, Kraków, Poland.

The aim of investigation was to recognize the interference between the natural environment and the human activity at the foreland of the western part of the Ukrainian Carpathians in the area of upper Dnister. This study focused on vegetation and land-use reconstruction of the last 7,000 years. Pollen analysis of fossil peat deposits have provided information about vegetation history, climate and human impact in the Dnister River valley and surrounding hills. Pollen analytical investigations were carried out in an archaeological context. On the base of six pollen diagrams, which together cover the period from the Mesolithic to the Middle Ages, different phases of human impact on the environment were detected and described. Radiocarbon dating have provided a chronology for the palynological events. It may be concluded that the studied area was used by early Neolithic tribes. It was associated with a decrease in forest areas in different scale. From the time of 2,500 years before present the agrophytocenoses and other anthropogenic plant communities provoked deforestation in the large scale.

P2409. Holocene snow avalanches and their long-term impact on subalpine vegetation in Tyrol (Austria)**J. N. Haas;**

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Snow and ice avalanches belong to the most destructive natural hazards in the Alps. However, the Holocene history and frequency of large avalanches as well as their long-term impact on vegetation was unknown up to very recently. Here we will present results performed in the Ziller Valley (Tyrol) within an interdisciplinary research programme between palynologists, dendrochronologists, foresters, modellers as well as avalanche experts (HOLA -

Evidence and Analysis of Holocene Avalanche Events', <http://bfw.ac.at/800/2089.html>) showing that avalanches did contribute to plant diversity changes at timberline for at least the last 6000 years.

Extrafossils such as algal cysts or fungal spores thereby clearly add to our understanding of (palaeo-)ecological change at high altitude and allow to assess the relative importance of climatic change and of prehistorical human impact in the Eastern Alps. Cysts from snow algae such as *Chlamydomonas* and *Chloromonas* may hereby help to reconstruct the long-term avalanche frequency as well as to quantify winter snow amounts for the Holocene.

P2410. Botanical investigations in a 6000-3370- B. P. highland rock shelter in Central Sahara

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The 6.035 ±100 and 3.700±40 B. P. layers in Uan-Muhuggiag rock shelter in Tadrart Acacus, Fezzan, have yielded a few plant macro-remains. Among these it has been possible to find wood belonging to Cupressaceae, Leguminosae, Oleaceae, Tamaricaceae and some fruit-stones of *Balanites aegyptiaca* and fruits of *Zilla spinosa*. These species look to be present in the area at the time as seen through pollen and macro-remains analysis led by many researchers. The man gathering plants could also collect plants far away the shelter during his outing the area. The preference he made clearly shows that prehistoric saharian native had a good understanding of use of wood and the properties of the plants. In the level examined up to now, the former, UM/A-1, besides sudanian species also shows a less amount of saharo-arabian species; in the older layer, UMb/2b, mostly sudanian species look to be present. Charcoal from different sites in eastern and central Sahara gives evidence that shifts to the north of tropical climate and vegetation belts happened in the Holocene.

P2411. The Late Neolithic settlement of Pestnacker, Lkr. Landsberg/Lech, Bavaria. Implications for the landscape - human impact and/or climate

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At the Late Neolithic settlement of Pestnacker, 20 km south of Augsburg, four superimposed settlement phases belonging to the Late Neolithic Altheim Culture were distinguished archaeologically on the border of a little valley. Phase Ia, established in 3496 BC, was partly destroyed by fire after four years and rebuilt (Phase Ib). After another 15 years the settlement was abandoned. Thirty years later Phase II began on the same site. The duration of Phase II is unknown due to poorer preservation conditions caused by artificial drainage of the valley since 1930. Therefore Phases III and IV are almost totally represented by carbonised plant remains alone. Altogether, approximately 300 taxa could be recognised. The most important crop was Emmer followed by Einkorn. A little barley and tetraploid wheat of the *T. turgidum/durum*-type were found in every phase. Flax, opium poppy and peas were cultivated in addition to the main crop. The gathering of wild fruit played a large role in nutrition. The detection of a dung-heap behind one of the houses and the large number of grassland species identified, together with pollen analytical evidence suggest the gathering of hay.

P2412. Rice and foxtail millet cultivation reconstructed from weed seed assemblages in the Chengtoushan site, central China

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Crop weeds have been successfully used for evaluation of farming practices in archaeological sites and reconstruction of the environmental condition. In rice agricultural sites in East Asia, however, a few studies of crop remains have been attempted. We evaluated the crop husbandry based on plant macrofossils including crop grains and weed seeds in the Chengtoushan site, Hunan Province, central China, which is one of the oldest rice agricultural site around the Yangtze River Basin. In the moat surrounding the site that is located on a loess plateau that juts out into the alluvial plain, we recognized three cultural layers during

the Daxi Culture. Plant macrofossils in silty clay deposits in the moat consist of abundant rice and foxtail millet grains with many weed seeds. Radiocarbon age of these fossils shows that rice and foxtail millet cultivation dated back to 6400 cal. years B.P. The weed seed composition characterizes farmland and ruderal environments in the site surrounded by the moat. We assumed foxtail millet and rice cultivation practiced within the site on loess plateau, along with a paddy style rice cultivation in the alluvial lowland outside of the site.

P2413. Regional Cartographic Models of Vegetation Cover

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Digital vegetation maps scale of 1: 2500 000, 1: 1000 000, 1: 200 000 for the North of the Russian Far East (Magadan Region, Chukotsky autonomous district, Korjaks autonomous districtas) are created. Polygons are shown on the map. Withing these polygons real vegetation complexes are shown. Vegetation complexes determined airvisually. Each map included in separate GIS project as the whole polygonal coverage. Data base of these projects includes information on each polygon: area, perimeter, index of predominant vegetation cover type, procent correlation of the main vegetation types in complex. Cartographic models are used in botanical researches for decoding remote sensing data, species plants communities distributions. GIS projects on reindeer ranges of Magadan Region, Chukotsky autonomous district, Korjaks autonomous districtas are elaborated also. Data bases of these projects contain information on season forage storages and reindeer range capacity.

P2414. Floristic and phytocoenological analysis of the ass. Lemnetum minoris Th. Müller et Görs 1960 (the Blace Lake, southern Serbia)

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The Blace Lake is located near the Blace town, in southern Serbia. Habitats of the ass. Lemnetum minoris are open water surfaces and the coastal zone of the lake, from which, during summer, water partially or completely retires. Such habitats are colonized with the meadow species. The ass. Lemnetum minoris was determined, based on ten phytocoenological relevés contain 66 species. The primary edicator of this community is *Lemna minor* L., while the characteristic species are: *Ceratophyllum submersum* L., *Lemna minor* L. and *Schoenoplectus lacustris* (L.) Palla. Ten floristic elements are registered, within the spectrum of the areal-types with the domination of the following: sub-eurasian (25.7%), cosmopolitan (13.7%) and Eurasian (13.6%). At the investigated locality seven life forms were registered. The dominant are hemicryptophytes (42.4%), geophytes (33.3%), therohemicryptophytes (10.6%) and helio-hydrophytes (6.1%). From the ass. Lemnetum minoris, the three subassociations are derived: typhetosum prov., lemnetosum prov. and caricetosum prov.

P2415. Karst mires in Tula region (Middle-European Russia)

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Mire inventory was carried out in Tula region, Russia (54°N 37°E). Different mire types were distinguished. They are valley fens, bogs and karst mires. Tula region is situated in the northern part of the Middle-Russian Upland where limestone structures dominate. Karst phenomenon is displayed by the development of dolin depressions which can be sometimes very deep. The depressions are often filled with ground water, and many of them have become lakes or ponds. Later, due to a process of overgrowing, some of them have turned into mires. Mires are formed through a paludification process as well as in a case of shallow depressions which collect surface water. Karst mires are small (<1 ha) and they have a clearly circular form. Very often they can join together due to the ongoing karst process and form "cascades". Key areas with a series of karst mires were studied in Tula city surroundings. Data on vegetation, pH of mire water, peat deposits, productivity of communities and microbiological activity were obtained. Large-scale vegetation mapping was made as well. The studied karst

mires are mainly mesotrophic (pH ~6). Vegetation communities are typical of forest taiga swamps.

P2416. Vegetation of fresh alluvial habitats

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The pioneer vegetation on pointbars of the Allier and Volga shows many similarities. However, there are also some clear differences related to: hydrodynamics, morphodynamics (sedimentation and erosion), and physical conditions (sediment characteristics). The structure of pioneer vegetation on pointbars of the Allier is more complex due to the variable composition of the bed material. The upstream coarse gravel part of the pointbar is generally almost without plant cover, or with some *Populus* or *Salix* seedlings. In the central part of the pointbar, with mixture of gravel and sand, communities with *Corrigiola litoralis* and *Portulaca oleracea* appear. On sandy areas, normally found in the downstream part of the pointbar, communities with *Eragrostis pilosa*, *Xanthium orientale* are present. The pioneer communities exist only for one summer season: during the next flood they are either covered by fresh alluvium, moved away with the flow, or develop into the next stage of succession.

P2417. Study of pteridophyte communities present in humid mountain forests of central Cuba.

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It is analyzed, for the first time in Cuba, the taxonomical composition and the structures, in the space and seasonal scale, of the communities of ferns and allied plants (pteridocenosis) integrated to different vegetation units. The chosen formations were: the cloudy closed forest, the mountain rainforest and the humid evergreen forest, located in altitudinal order in the north hillside of "Teta de Juana", hummock elevation of Sancti Spiritus's mountains. Two aleatory samplings were made, one in dry and another in rainy season. Was registered 64 species. The taxonomical composition of the communities was evaluated by means of indexes that measure: the species richness, the heterogeneity and the evenness. The structure was studied starting from the analysis of the biological spectrum. It was also carried out a nodal analysis. Significant space differences were observed in the taxonomical composition and the structures of the pteridocenosis settled down in the three habitats, being the highest values in species richness and heterogeneity in the rainforest, followed by the evergreen forest.

P2418. Floristic and fitocenological characteristics of pyramidal european silver fir (*abies alba* var. *pyramidalis* Carr.) in southwest Serbia

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The natural stand of pyramidal silver fir at Ogorijevac locality (at Pešterska visoravan) is the unique fragment in Europe because of stand floristical and fitocenological characteristics, as well as morphological, anatomical and other characteristics. Pyramidal European silver fir (*Abies alba* var. *pyramidalis* Carr.) is characterized by the branches which leaves the made stem under an acute angle, where as with age, the angle of branch insertion increases and the shoots growing on these primary branches are negatively geotropic. The number of leaves on the shoots of pyramidal European silver fir is highest than that of European silver fir. The former leaves are radial arranged all stem, and the latter lie in a horizontal plane sideways. Ecological indexes for pyramidal silver stand are developed and compared with ecological indexes of stands in which this form of fir is not present.

P2419. The cryptogamic flora of the Zgurasti Cave and surroundigs. (Apuseni Mountains, Romania)

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Hungarian Academy of Sciences, Eger, Hungary.

The Zgurasti Cave lies in the Bihor Mountains in the Romanian Western Carpathians. In fact it is 35-40 m diameter and 50 m deep pithole with a cave joining to it, carved in limestone. In the cave there is a temporary underground lake on clay ground. In spite of the fact that the cryptogamic flora of Bihor Mountains is relatively

well investigated, till now the cyanobacteria, lichens and bryophytes were not reported from this peculiar carstic depression. We could provide new data to the floristical knowledge of the studied area. It is the first time reported from Bihor Mountains *Ptilidium pulcherrimum* (Weber) Hampe, *Tritomaria exsecta* (Schrad.) Loeske. Other interesting species are *Anastrophyllum hallerianum* (Nees ex Lindb.) Schust., *Nowellia curvifolia* (Dicks.) Mitten, *Ditrichum crispatisimum* (Müll. Hal.) Paris, *Orthothecium rufescens* (Brid.) B.S.G., *Rhynchostegiella tenella* (Dicks.) Limpr.

P2420. Floristic composition in the Aragua River Basin, Municipio Piar, Monagas state, Venezuela.

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Explorations made during five years provide information on the floristic composition of a little studied area, between the Llanos and the Coastal Cordillera (eastern sector) regions (10° 00' N, 63° 20' W, 320 - 580 m elevation). 528 spp., 361 genera and 107 families were identified. The most diverse families include Fabaceae, Euphorbiaceae, Asteraceae, Mimosaceae, Caesalpiniaceae, Rubiaceae, Poaceae, Bignoniaceae and Malvaceae. The area is covered by a mosaic of savannas and heterogeneous forests. The savanna (97 spp.) occur on the slopes and some hilltops (400-580 m), with low fertility sandy or rocky soils; tree and shrub savannas occur in shallow depressions with poor drainage. The gallery forest (107 spp., where evergreen trees dominate, also lianas and epiphytes occur) are located on flat or gently undulating fertile soil plains with stony profiles, adjacent to the Aragua River or its effluents (320-420 m). Deciduous forest (103 spp.), predominate in the valley or are found as secondary vegetation in logged and farmed areas. This zone has a rich vascular plant flora, with 53 species not represented on the adjacent floristic regions.

P2421. Local scale variation in plant community attributes in the Semi-arid region, Northeastern Brazil

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At local scale, variations in floristics and structure associated to abiotic factors are reported for the typical vegetation of Northeastern Brazil semi-arid, Caatinga. We tested if six adjacent biotopes with distinct altitudinal (450-690m) and pedological aspects present different woody plant attributes of structure and physiognomy. In each biotope, a 20 x 50 m plot was established, divided into 10 sub-plots. Maximum distance between sample plots was 7 km. For each biotope, relative density, basal area and frequency were calculated for all families and species. Based on results of floristics and structure of plant communities in each biotope, two matrixes were created: one based on presence/absence of species by sub-plot and other with number of stems per species in each sub-plot. A total of 2,055 stems, 23 families, 64 species was recorded. Clear altitudinal or pedological local-scale gradient could not be defined along *a priori* defined biotopes, which hold in fact two phytocoenoses: Plateau Summit, at greatest altitude, with distinct physiognomy and exclusive taxa; and lower altitude community, presenting physiognomical and structural similarity.

P2422. The relation of present vegetation and environment at Chipei Island

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Low elevated Chipei Island, lying northern Penghu Archipelagoes, constituting of basalt at east half and sand beach at west half, is primarily covered with herbaceous vegetation. Based on the investigation of 113 homogenous sampling areas of each 2x2 m², 90 native, 25 naturalized and 12 cultivated species were recorded including 40 families, 101 genera and 127 taxa. By the TWINSPLAN, the vegetation could be classified to 4 types such as (1) afforestation at southwestern part to avoid the winter gale, (2) bushes at southern slope or indented abandon farming areas, (3) wildspread grassland, (4) sand beach vegetation at northeastern and western coasts. The herbaceous vegetation near the tidal coast was dominated by the native *Zoysia matrella* and it's hardly invaded by introduced species. With low coverage of vegetation at

western broad Golden Coast, its majority consisted of *Ipomoea imperati*, whose habitat area is however the greatest in Taiwan. An unstable sand beach under severe environmental stress due to the assailing by northeastern winter gale and corrosion by the ocean current is a characteristic for Chipei's vegetation.

P2423. Treeline Vegetation of the River Chirukhistskali (South Colchis, Georgia)

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The paper presents the survey of the treeline vegetation of the high mountainous region of the river Chirukhistskali (South Colchis, Georgia). The major part of the treeline vegetation of the area is experiencing strong anthropogenic and zoogenic pressure. However, it is noteworthy, that such undamaged communities as *Festuco-Piceetum* and *Fago-Betuletum* have remained here. The associations *Abieto-Piceetum*, *Rhododendro-Piceetum* occurring in the region are distinguished by great specific diversity and forests of *Picea orientalis* by abundance of endemics.

We consider it essential to take appropriate nature protective measures to avoid landslides, mud-streams and avalanches in the studied area. It is also necessary to allocate protected territories, prohibit overgrazing and deforestation for obtaining firewood as well as extend haymaking areas at the expense of pastures.

P2424. Syntaxonomy of the Northern Black Sea Region vegetation

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An area of the Northern Black Sea Region covers unique natural landscapes (2 mln ha) created as a result of fluvial and sea activities. In consequence of enlarging human impact the problem of diversity conservation in the industrial and agrarian south of Ukraine is the pointed one. According to the UN Convention on biodiversity conservation the Institute of Botany, the National Academy of Sciences of Ukraine within the international scientific cooperation executed the work "Syntaxonomical diversity of psammo- and halophytes of the Eastern Mediterranean within Ukraine". Vegetation's classification of the region includes 24 classes, 37 orders, 59 alliances, 274 associations, among which 60 are meadow-wire, 55 - aquatic, 40 - psammophyte, 19 - salted, 47 - saline marsh, 38 - violated, 46 - occur only in area of Ukraine, 3 are the new. The prodrome is a stage of the project of the Formation Program of European Econet of the seaside National Azovo-Black Sea ecocorridor. The next tasks of researches are to study communities' dynamics, to map vegetation in detail, to do monitoring and management of phytocoenoses, including of rare syntaxa into the Green Data Book of Ukraine.

P2426. Inter-annual variations in the population structure of an herbaceous community of *Caatinga* vegetation in Pernambuco, Brazil

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The influence of climatic variations on the herbaceous component of the *Caatinga* vegetation was examined in the state of Pernambuco. A total 35 1x1m-plots were in a level microhabitat, 35 in a riparian microhabitat, and 35 in a stony microhabitat were established. During two consecutive years all of the herbaceous plants in these plots were counted, measured and collected. The density, frequency, dominance, importance index and Shannon-Wiener diversity index for each species was calculated for each year. The herbaceous flora was composed of 71 species. The families with the greatest species richness were Malvaceae, Euphorbiaceae, Poaceae, Convolvulaceae, Fabaceae, and Portulacaceae. The diversity indices were 2.66 and 3.01 nats.ind⁻¹ in 2002 and 2003, respectively. Annual seasonal climatic variations greatly modified these populations structure emphasizing the fact that long-term studies are needed in order to better understand the dynamics of the herbaceous component of the *Caatinga*.

P2427. Current situation of flora of "Puerto Viejo" Wetlands, Lima - Peru

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"Puerto Viejo" wetlands are located at the south of Lima (71 km toward south), in the province of Cañete and district of San Antonio de Mala (12° 34' 39" LS and 76° 42' 21" LW), with an area of about 200 ha. This is supplied of water from Mala River, but it became salty. There are many pools with different depths. The vascular flora is composed for 31 species, 29 genera and 17 families, where Magnoliopsida has 21 (67.74%), Liliopsida has 9 (29.03%) and Pteridophyte has 1 (3.22%). The most representative families were Poaceae, Asteraceae, Chenopodiaceae, Amaranthaceae, Cyperaceae, Fabaceae, Solanaceae; with predominance of herbaceous plants (90.32%) and shrubs (9.67%). Also it was considered forms of life, so pterophytes has 32.5%, hydrophytes and helophytes 29.03%, hemicriptophytes 19.35%, camephytes 19.35% and nanophanerophytes 3.22%, and we identified 6 plants communities: aquatic plants, bulrushes, reeds, cyperaceous, shrubs and lawns.

P2428. Sustainability of *Elaeodendron transvaalensis* harvesting in Venda region, Limpopo province.

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Several years earlier, the Chiang Mai Declaration had noted that, since medicinal plants form the basis of medicines used by the majority of the population of most developing countries, the loss of certain medicinal plant species and reduced supply of other important plants would have a direct impact on human health and well being.

Elaeodendron transvaalensis is one of the medicinal plants used very often by people in the Venda region. It is known to treat a variety of diseases. The diseases treated by the plants include; cleaning of stomach from any disorder, treatment of ulcers, treatment of venereal diseases (STDs), and treatment of fungal infections.

Due to its wide usage by traditional healers it has found its way into the muti markets and it is amongst the seven most commonly traded plants in the Venda region. The study investigates the impact of bark harvesting on this species.

Bark harvesting for medicinal purpose is the major contributor to the loss of *E. transvaalensis* since people only utilize it for firewood when it is dry. Cultivation intervention is therefore necessary to reduce the stress experienced by *E. transvaalensis*.

P2429. Phenological analysis and land cover classification for the Papaloapan watershed vegetation using MODIS imagery, México.

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The most recent technology to evaluate vegetation status includes the use of satellite images derived from sensor MODIS (MOD13 product). It contains EVI and NDVI indices used for the phenological analyst. NDVI indices have been intensively used from 80s with AVHRR imagery. Nevertheless, their spatial resolution spatial (~1.1km) of this images is low compare with ~250m of MODIS sensor. Additionally, the applied MODIS enhanced narrow bands, canopy background and atmospheric corrections, and also applied the BRDF. So, we generated sequential images from years 2000 to 2004 with monthly images with the MVC technique. For the phenological analysis, a dimension reduction technique, based on principal component analysis (PCA) was then applied. The first five components of a PCA on annual series (12 monthly images) explained ~91% of vegetation variability and were used for an unsupervised classification (ISODATA) obtained 60 classes. From each class averages of sequential images a classification was produced using cluster analysis. From these procedures a final new classification was produced to represent information classes into land cover categories.

P2430. Widespread Of Pine Pine *Pinus peuce* Grisebach, 1839 On Pelister And Surrounding Mountains

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Pinus peuce Grisebach, 1839 was found in Pelister 1839 by Austrian botanist August Grisebach. *Pinus peuce* is widespread in surrounding hills and mountains as Ilinska, Bigla, Plakensko-Snegovski massif. Also exist two stunted examples of this pine in Bitola at 650m altitude.

Now exist examples of this pine in Bitola at 650m altitude.

On the *Pinus peuce* tree are nested the following birds: *Fringilla coelebs*, *Carduelis chloris*, *Parus ater*, *Columba palumbus*, until the following bird eat seed of pine pine: *Passer montanus*, *Loxia curvirostra*, *Serinus serinus*, *Coccothraustes coccothraustes*, *Pyrrhula pyrrhula*, *Carduelis chloris*, *Carduelis spinus*, *Carduelis carduelis*, *Fringilla coelebs*, *Fringilla montifringilla*, *Parus caeruleus*, *Parus major*, *Columba palumbus*, and mammals: *Sciurus vulgaris* and *Apodemus sylvaticus*. As a result of global warming 2002 in spring months by snow weith many thousands trees were claming down and damages.

P2431. Postfire reproductive dynamics in *Pinus halepensis* (Aleppo pine) forests of northern Euboea Island, Greece

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Pinus halepensis is a typical Mediterranean pine that has evolved several adaptive traits in response to fire. It is an obligate reseeder and its perpetuation depends on both the yearly production of prolific seed crops and the safeguarding of a fraction of seeds within serotinous cones. The reproductive dynamics of even-aged, Aleppo pine populations were studied in three forests of northern Euboea Island (Gouves, Rovies and Pili) burned in 1994, 1996 and 1997, respectively. In each forest, four permanent observation plots were established (summer 2004) at randomly selected sites and a number of pine saplings were tagged for long-term monitoring. Extensive field measurements of pine sapling density, individual growth (height, canopy size) and reproductive capacity were carried out at the end of the 2004 growing season. It is noteworthy that a significant fraction (35.8, 32.4 and 26.2%, respectively) of each pine population had already produced cones in close correspondence to both their age and average height. In addition, mature cones from each population were collected and data on cone, seed and germination characteristics were obtained in the laboratory.

P2432. Floristic diversity in silver fir - beech forest on Croatian karst explained with ecological and forest stand structural factors

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Silver fir - beech forest represents almost continuous area of 3000 km² making it the most widely distributed altimontane forest on Croatian karst. Its syntaxonomical status is still not completely resolved. To give further insight into its ecological and floristic characteristics, survey across complete areal was carried out. Sample consisted of 151 plots (cca. 500m²) with two smaller ones (cca. 80m²) positioned inside each of them, i.e. 302 small plots. Data about floristic composition of over 200 species, available light (measured with hemispherical photographs), stand structures (e.g. increment, crown damage, volume) and geographic coordinates were sampled in the field. In frame of the GIS, data about altitude, climatic variables, and DEM derived variables (e.g. slope, aspect) were sampled. Using multivariate analyses (cluster and ordination analyses) several distinct groups were identified based on their floristic profile, with most important ecological and forest stand structural factors recognized.

P2433. Fragmentation and biodiversity. An approach to the structural characterization of the vegetation of the council of Vila Real (Northern of Portugal)

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The area selected in this work is located in the municipality of Vila Real (North of Portugal). Based on this vegetational differentiation and on the successional process from pioneer states to the climatic ones, eight types of communities were selected: one tilled and ruderal communities, three shrubby communities and, finally, three forest communities. Fifty five relevés were distributed per the eight different types of vegetal communities. The three matrices were oined in a contingency matrix to analyse the information derived from the different basic structural matrices. In the present case 34 contingency variables were proposed. The results obtained indicate a very important "landscape connectivity", as a capacity of connectivity among the different types of communities and inside each of them. These floristic-structural combinations will be significantly based on the generalist species (*heterogeneous undivided habitat* effect). The access of the generalist species will improve floristic-structural combinations: the anthropic activities must be considered in the preservation of the biodiversity presented in the agroecosystem.

P2434. Complex research on the flora, habitats and traditional land use of Putnok Hills (North-East Hungary)

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Valuable plant taxa found in the Putnok Hills (Northern Hungarian Mountains) and unique for a bigger area can be preserved in their original habitats by sustaining traditional agricultural management patterns. Old agricultural activities on diverse habitats resulted in specially structured landscape mosaics. Regularly mowed wet meadows along the streams maintained large sedge communities with *Carex cespitosa* and *C. buekii*, tall herb fringe communities with *Inula helenium* and molinia meadows with *Iris sibirica*, *Orchis laxiflora* subsp. *elegans*, *Dactylorhiza incarnata* and *Eriophorum* formations preserve *Orchis morio*, *Dianthus deltoides* and *Platanthera bifolia*. Regularly mowed lawns of extensively cultivated orchards hide *Aster amellus*, *Iris aphylla* subsp. *hungarica* and *Orchis purpurea*. Valuable species in abandoned grapeyards are *Pulsatilla grandis*, *Polygala major*, *Linum falvum*, *Orchis tridentata* and *Stipa pennata*. Extensively cultivated small parcels of arable land give home for rare weed species such as *Agrostemma githago*, *Euphorbia salicifolia* and *Rapistrum perenne*.

P2435. Vegetation survey of *Quercus pubescens*-stands N of Graz (Austria)

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Xerothermic vegetation-complexes including stands of *Quercus pubescens* s. l. (downy oak) located on S-exposed slopes of a calcareous hill chain on the fringe of the Eastern Alps N of Graz (Styria, Austria) have been studied. The vegetation mosaic includes the associations Geranio sanguinei-Quercetum pubescentis, Ligustro-Prunetum, Cotoneastro-Amelanchieretum, Peucedanetum cervariae and Seselietum austriaci. These complexes are surrounded by stands of Cyclamini-Fagetum, the dominant forest type in the investigated area. 163 relevés have been processed using the Braun-Blanquet approach. A digital vegetation map based on aerial photographs (scale 1:2800) has been produced with ArcView GIS 3.2 software. In the course of our studies, some of the xerothermic patches have been nominated as a NATURA 2000 area. The species-rich communities characterized by a mixture of submediterranean and dealpine elements are endangered by succession as well as the invasion of the alien species *Robinia pseudacacia* and *Ailanthus altissima*. A synoptic table has been prepared to elucidate the synchorological and synsystematical position of the downy oak-stands N of Graz.

P2436. Change of the species composition of the flora Karelian Isthmus (Leningrad region)

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Karelian Isthmus is territory in the North-West Russia between Finland gulf on the west and Ladoga lake on the east, river Neva

on the south and Finland and Karelia on the north (15 000 km²). Check-list include 1186 wild vascular plants (817 aboriginal species) from 114 families and 114 genera. The own field investigations in 1994-2004, work in Herbariums (LE, LECB, WIR, H, HSI, KUO, TUR) exposed the changes of plant diversity for 160-year period with beginning at first herbarium collections. The work include characteristic of nature condition and vegetation, systematic structure, geographical, ecological-biological (life forms, dispersal, phytocoenological structure) aboriginal flora, classification of the adventive elements, post-glacial development and characteristic of the threatened plants of the flora, floristic division into districts, maps of distribution for 467 species.

P2437. The Effect of Elevation, Slope and Geographic Aspect on Plants Biodiversity in Caspian Beech Forests

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Biodiversity covers the number, variety, and arrangement of living organisms. It is typically described, quantified, managed, and used for various living organisms groups. Biodiversity is affecting by environmental factors such as elevation, slope and aspect. Sixty permanent sample plots with an area of 1000 m² and per plot including 5 micro plots with 9m² surface were selected in two types of *Fagetum* and *Fageto-Carpinetum* in Nowshahr research forest in the north of Iran. The woody plants biodiversity were considered in every sample plots by the use of current indices and the regeneration were inventoried in micro plots. The results show negative and significant correlation between biodiversity indices and elevation that it maybe related to temperature decrease with elevation. There is a significant and positive relation between beech regeneration and elevation. The slope effect was not significant on regeneration and woody plants biodiversity but the aspects effect was different in various indices. The total results show the effect of elevation is more than aspect and the effect of aspect is more than slope on biodiversity and regeneration in beech forest.

P2438. The Study of Natural Regeneration of Woody Species after 15 years in Alder Plantation Site in Mazandaran Forests

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In the lower forests of wood and paper industry forest in Mazandaran province four sites of 15 years old of Alder plantation as well as one site of witness forest was selected for the study of regeneration of woody species in planted site after 15 years. In each site three plots 20x20m were selected. The number of woody plants was counted, diameter and height of generated species was measured in each plot. Species diversity was examined by using of Shanon-Wiener function index as well as evenness and richness by using Simpson and Menhinic indexes.

The results of study show that 8 tree and shrub species observed in planted sites. The amount of diversity index in one planted site was higher while in other three sites was lower than natural forests. The evenness also in one planted site was higher than natural forest while in three other sites it was lower than natural forest. The species richness in tow planted sites was higher than natural forest.

P2439. The Nature Reserve Park „Heidenreichstein“ in Lower Austria: An example for conservation, management and economic significance of a regenerating peat bog

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The Northern Waldviertel in Lower Austria contained many peat bogs by reason of a suboceanic climate combined with Granite or Gneiss as bedrock. Most of the bogs were either turned into farm land or cut over for fuel production. Only very few sites remained and one of them, the "Heidenreichsteiner Moor", was protected in the 1970s and became a nature park. The land owners, a forest

co-operative, built a pathway for visitors with information boards about values and functions of the mire and the surrounding forest. A wooden platform and a watch over were constructed to enable people to see the beauty of the site either from the ground or from above. In a nearby museum, people can get additional information about peat land ecology, conservation issues and threats.

Taking this as an example, we present in a scientific film the ecosystem functions together with possibilities of mire management and protection and strategies to make a nature park of this kind economically successful and accessible for people. We show the measures of nature park managers to maintain the integrity of the habitat and to improve its infrastructure as a recreation area and a place to learn more about nature.

P2440. Pasture flora on family farms in Istria (Croatia)

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Autochthonous vascular pasture flora was researched at the locality of family owned farms which breed sheep, in Istria, Croatia. On Istrian agricultural farms, a total of 328 plant taxa (species, subspecies and varieties) were found. The taxa belonged to 200 genera and 47 families. Part most of them were *Compositae* (16.5%), followed by *Gramineae* (14.6%), *Leguminosae* (10.9%) and *Labiatae* (9.5%). It should be noted that pasture flora in Istria supports numerous species that are rare or endangered, for example those from the *Orchidaceae* family, in addition to species of the genus *Narcissus*, *Crocus*, *Helleborus*, *Dianthus* etc.

These results as preliminary investigation into the pasture flora may play as a guide for the management of pastures and pasture vegetation on family farms in Istria, which contributes not only to the full utilization of the pasture, thus advancing livestock production, but also to the plant preservation of pastures.

P2441. The Rangelands of Iran

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Rangelands are sometimes defined as unimproved grasslands, shrublands, savannas, deserts, and tundra. They occupy 51% of the earth's land surface and contain about 36% of the total living and dead plant carbon. Small changes in extreme temperatures and precipitation have disproportionately large effects in these regions because of the vulnerability to water availability and water balance. The rangelands of Iran comprise all of that area which is grazed extensively by livestock according to the tradition of common law and the peoples ancestral rights. This includes mountains, foot-hills and plains hacking natural vegetation of herbs, grasses, fords, and bushes. Iran comprises of 165 million hectares of which about 90 mil. ha. (54.5%) is rangelands. The remaining 75 mil. ha comprise 23.8 mil. ha. of agriculture land, 12.2 mil. ha. of wood land, 35.3 mil. ha. of Desert & degraded lands, 307 mil. ha. of inland water bodies; urban & rural areas and others. The rangelands of Iran are classified three classes according to vegetation cover.

P2442. Steppes in Towns - Naturalistic planting design in open urban space

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During the past decades the scientific approach to planting design was determined by the use of plants in accordance with typical habitat sites.

Habitat interrelated to garden sites means to consider a wide range of components such as local aspects and requirements of single species or individual development and influence of plant communities in suitably defined habitats. Contemporary understanding of nature and the tendency to conserve and manage it brings us to a new understanding of gardens. Naturalistic gardening has begun to be accepted as an aesthetic value in open urban space.

Due to the location in the Pannonic climatic zone, Vienna's townscape is predestined for naturalistic planting design - in accordance with the surrounding native herbaceous vegetation as Steppe Sites and Xerotherm Vegetation and with the need of low maintenance ecological planting design in open urban space. In the year 2000 the group for Plant Use and Planting Design (BOKU) started a project in cooperation with the local authority (MA 42), with the aim to establish Pannonic Plants in a public

garden. Theoretical background, experiences and results will be shown in a poster presentation.

P2443. Introduction of exotic Australian ectomycorrhizal fungi with eucalypt plantations and its spread to native potential hosts in the Iberian Peninsula

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Here we describe the introduction of Australian ectomycorrhizal fungi with plantations of eucalypts in the Iberian Peninsula, and whether these fungi can spread to potential native hosts. When we studied the diversity of ectomycorrhizal fungi in eucalypt plantations, we found fruit-bodies of ectomycorrhizal fungi specific to Australasian trees; such as *Hydnangium carneum*, *Hymenogaster albus*, *Hysterangium inflatum* or *Setchelliogaster rheophyllus*. Mycorrhizas of other exotic ectomycorrhizal fungi (unable to fruit outside its natural geographic range) were identified using molecular tools (RFLP-T and DNA sequencing of the ITS regions of the nuclear rDNA). We assessed then the potential spread of such Australian ectomycorrhizal fungi to native ectomycorrhizal plants. We found that exotic fungi spread beyond the eucalypt plantations depends on their compatibility with native hosts; i.e. of the Australian species *Laccaria fraterna* (= *L. lateritia*), which was found in Mediterranean shrub-lands in association with roots of the ectomycorrhizal shrub *Cistus ladanifer*.

P2444. Research on the drought-resistant capabilities of 47 lianes for quarry reclamation

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In order to apply Liane to greening abandoned quarries' slopes, the drought-resistant capabilities of the selected Liane species were tested. This research uses water physiological indexes, including temporary wilting ratio and leaflet water-holding ratio, so that the drought-resistant capabilities of the test varieties can be understood. The results show that the "hundred-mark system" synthetic evaluation method at the base of temporary wilting ratio and leaflet water-holding ratio can accurately reflect the drought-resistant capabilities of the Lianes, because of the results coinciding with that of the observation. The following Lianes were identified as drought tolerant and thus useful in land reclamation efforts: *Pereskia aculeata* Mill., *Parthenocissus heterophylla* (Bl.) Merr., *Macfadyena unguis-cati* (L.) A., *Bauhinia corymbosa* Roxb., *Ficus Pumila* L., *Thunbergia grandiflora* Roxb., *Cryptolepis sinensis* Merr., *Ficus tikoua* Bureau, *Tetrastigma obtectum* pl., *Tetrastigma jinxiuense* C.L.Li., *Tetrastigma caudatum* Merr.-et chun, *Ampelopsis cantoniensis* PL., *Ampelopsis heterophylla* var. *kulingensis* C.L.Li., *Pueraria montana* Merr.

P2445. Pseudoendemics and endemism mania (some examples from Cruciferae)

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There is temptation describe isolated populations as new endemic species. In Cruciferae this tendency appeared in case with *Schivereckia* Andr. described from *Podolia* (*S. podolica* Andr.). Each isolated population in Ural and Privolzhsk Hills were described by M. Alexeenko as separate species (5 species for Russia). In fact in Russia just one species occurs: *S. hyperborea* (L.) Berkut. There are 8 genera and 115 species endemic Cruciferae in "Flora of China". "More material- less endemics" - this assertion can be illustrated by the following: over 80 years genus *Borodinia* N. Busch considered as endemic of Eastern Siberia and Russian Far East. *Arabis alashanica* Maxim. described from China and considered as endemic of China for over 120 years is identical *Borodinia tilingii* (Regel.) Berkut. and as a result of taxonomic revision *Borodinia* was placed in *Arabis*. The type *Draba macrophylla* Turcz. was revealed by D. German. *D. macrophylla* is earliest description of species referred before *Borodinia*. New combination in the genus *Arabis* will be published in *Novitates Systematicae*. Taxonomic revisions on wide base can reduce the number of pseudoendemics in Russia and China.

P2446. The environmental conditions of the development of Nymphaeid-dominated habitats

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In the course of our research we have been searching for the answer to the question what are the major environmental conditions that determine the development of nymphaeid-dominated habitats. We have compared the hydrobotanical data of habitats with the results obtained from hydrogeological, geological and geophysical data. On the grounds of hydrogeological data we have found that (1) the nymphaeid-dominated habitats can all be spotted in the discharge zone of groundwater. In the course of geophysical examinations we compared the gravity lineaments derived from the digital data-processing of gravity data with the data showing the territorial placement of nymphaeid-dominated habitats. On the basis of this we could establish the following: (2) In every case the habitats can be found along the boundaries of gravity causative bodies (from Bouguer anomaly map), and they are on the 'brink /boundary' of the geological tectonic grabens. (3) Under each nymphaeid-dominated habitat we can find tectonic or micro- and macro-structural lines, or the development of the habitats can be directly related to them.

P2447. Application of fluorescence induction parameters to assess the environmental adaptability of endemic plants on the island of Crete

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Certain plant species of the families Campanulaceae, Dipsacaceae and Valerianaceae exist as endemic ones on the island of Crete. In order to screen the environmental adaptability of these plants, fluorescence induction kinetics were recorded on the field and then processed by JIP-test analyses. Additionally to the quantum yield of photosynthesis (F_v/F_m), specific fluxes and activities of the photosynthetic apparatus were estimated. We present data concerning the reaction centre density, the photosynthetic antenna size, non photochemical quenching constants and other data that allow for a comparative study on the overall performance of these species in their natural environment. First evidence shows that most of these endemic plants experience stress conditions in the natural environment of Crete, such as high solar irradiation, meaning that probably they are not properly adapted to it.

P2448. Ecophysiological responses of some dune species to experimental burial under field and controlled conditions

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Field, greenhouse, and growth chamber experiments were conducted to determine the effects of different burial treatments on photosynthesis, chlorophyll-a fluorescence, leaf area, biomass, leaf thickness, chlorophyll content and chlorophyll a/b ratio of nine sand dune species: *Cakile edentula*, *Corispermum hyssopifolium*, *Strophostyles helvola*, *Cirsium pitcheri*, *Elymus canadensis*, *Oenothera biennis*, *Xanthium strumarium*, *Agropyron psammophilum*, and *Panicum virgatum*. Although there were significant differences between the species, all of them exhibited stimulation in growth following burial in sand. However, this stimulatory response lasted only for the first two to three weeks after burial and then leveled off or declined. The positive effects of burial were more pronounced in the two perennial grasses, *A. psammophilum* and *P. virgatum* due to higher energy content in roots, rhizomes, and underground stems. The main reasons for the stimulation in growth were, increase in leaf area, leaf thickness and CO₂ exchange rate post-burial. The total chlorophyll content of the leaves of buried plants of the two perennial grasses were higher than the controls.

P2449. Selection of elite poplar clones for phytoremediation of soil contaminated by heavy metal: in-field and glass-house experiments.

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In the framework of a project on the use of poplar for phytoremediation, three large-scale experiments were set up. During the spring of 2003, 140 clones (12 cuttings per clone) of *Populus alba*, *P. nigra* and commercial hybrids were planted on an area contaminated with Cu and Zn. Five clones, selected according to survival, were evaluated for metal and polyamine content. They were used to set up a new experimental field the following year. Survival, photosynthetic activity, metal content and mycorrhizal status were evaluated. The clones Villafranca and Jean Pourtet, inoculated with the mycorrhizal fungi *Glomus mosseae* and *G. intraradices*, were grown in a glasshouse on soil supplemented with Cu and Zn. Survival, photosynthetic activity, morphometric parameters, mycorrhization, metal content and soil remediation were evaluated. Results provide information on the best performing clones for phytoremediation, on metal accumulation and translocation, on polyamine variations under metal stress conditions, and on the effect of mycorrhiza on several of these parameters.

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P2450. Growth phenology of *Cenchrus ciliaris* L. accessions established in experimental station in the south of Tunisia

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Objective of this work was to study the growth phenology of *Cenchrus ciliaris* accessions (A1, A2, A3, A4), growing in the south of Tunisia. The measured parameters were number and length of leaves on the stem, leaf elongation and number of spikes per individual. A principal component analysis (PCA) was performed to get a synthetic appraisal of the whole data set. The main results shown that all the studied accessions have a bimodal growth season. Autumn growth was much less important than the spring growth. Main leaf and spike number were less in the autumn than in spring. An important leaf elongation was observed during the first growth period with respectively 19, 12, 16 and 16 mmj⁻¹ for A1, A2, A3 and A4. For the second growth period, leaf elongation is less important for all accessions. In PCA, A2 and A4 were situated near the origin and better correlated with axis 1, have an important leaf number. However A1 was located at positive extremity of the axis 1. It is characterised by a great spike number. Our results show that this species appear to be adapted to arid region.

P2451. Study of competition between purple nutsedge weed (*Cyperus rotundus*), bermudagrass (*Cynodon dactylon*) and ryegrass (*Lolium perenne*) lawns

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Effect of purple nutsedge densities and proportions on the intraspecific and interspecific competition of the weed with bermudagrass and ryegrass lawns using the replacement series design was studied. Pure and mixture stand densities were prepared as 4, 8 and 16 plants per plot. The proportions of lawn species: nutsedge were 100:0, 75:25, 50:50, 25:75 and 0:100. Height, leaf area and dry weight of each species and the following indices: relative yield, relative yield total, aggressivity, relative crowding coefficient, relative competitive ability index were assessed. The results showed that height, leaf area and dry weight of lawns significantly decreased with increasing of nutsedge proportion in mixture. Relative yield of bermudagrass was more affected by interspecific competition compared to nutsedge. Competitive index of nutsedge in all mixture proportions was greater than bermudagrass. With respect to relative crowding coefficient, nutsedge was more competitive compared to bermudagrass. Competitive index quantities of nutsedge were greater than ryegrass. Ryegrass was more susceptible to nutsedge interference compared with bermudagrass.

P2452. Effects of environmental and competitive stress on vegetative growth and resource storage: Causes for the low competitive ability of three endangered floodplain species

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The competitive abilities of three endangered European floodplain plant species *Cnidium dubium*, *Gratiola officinalis* and *Juncus atratus* were compared by growing each of them in a pot with a phytometer species, *Agrostis stolonifera*. Four different environmental conditions were applied, representing a variety of habitats in the river wetland.

As expected these 3 species tested had little or no negative effect on the growth of the phytometer. The reverse situation was found. *Cnidium*, *Gratiola* and *Juncus* showed a strong negative response to competition.

Beside the classical competition experiment our studies were also designed to address some physiological factors which may be responsible for poor competitive ability of the three species. Both carbohydrate and nitrogen metabolism were investigated.

For example the pool of free -amino acids was partly affected by high competition intensity. The maintenance of its integrity is crucial, as it acts to insure the delivery of nitrogen to protein metabolism.

P2453. Ecotypic variation in competitive abilities along a rainfall gradient in Israel

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The mechanisms of interaction between plants along productivity gradients have been the subject of an intense debate. This study approaches the debate from an intraspecific perspective, using ecotypes of three annual species found along a rainfall gradient in Israel. The experiments examine if ecotypes are differentially adapted to the presence of neighbors, and which are the life stages at which plant-plant interactions are most influential. We predict that plants at the dry end are facilitated by neighbors and therefore, invest rather in adaptation to the harsh climatic conditions. On the other hand, plants at the wet end should primarily invest in above-ground vegetative growth in response to competition. Some of our trials will simultaneously test whether plasticity of traits in response to contrasting conditions of water availability and presence of neighbors changes predictably along the gradient. Our first results partly support our prediction, though the responses were highly species-specific.

P2454. The influence of neighboring herbaceous plants on survival, growth and biomass production of Scots pine seedlings

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The effect of herbaceous species, dominating in early succession stages on abandoned fields, on planted Scots pine seedlings survival, growth and biomass production was studied in 2-year field experiment. The experiment was carried out in three synusiae dominated by morphologically different species: *Hieracium pilosella*, *Festuca rubra*, *Elymus repens*. Four types of competition were applied as a treatments: a) full competition: below- and aboveground, b) only aboveground, c) only belowground, and d) no competition. I hypothesized if the fitness of Scots pine are determined by competition, then there would be high variation between different treatments and synusia types. All measured parameters were clearly affected by applied treatments in all synusiae. Scots pine seedlings did well in a treatment with "no competition" and synusium dominated by *Hieracium pilosella* and much worse in synusiae of grasses. Both above- and belowground competition strongly affect Scots pine seedlings, what was especially true for species with strongly developed root systems.

P2455. Nursery phenological observation of different Douglas-fir provenances

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Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) is one of the most productive, coniferous species of North America and Canada and one of the most popular introduced species in Europe. Its

diversity is conditioned by the width of its natural range of distribution, and the genetic variability and ecological adaptation are directly dependent on the provenance origin. The selections of introductions species need provenance testing in new environmental conditions.

Douglas-fir is a species very susceptible to early autumn and late spring frosts. Bud burst phenology is closely associated with adaptation, survival and tree height growth. The seedlings produced from original Douglas-fir seeds originating from British Columbia, Canada, were the initial material for nursery phenological observation of different Douglas-fir provenances in Serbia. Three years of phenological observation is the aim of this paper.

P2456. *Dioon purpusii* Rose population structure analysis in three sites of Oaxaca, Mexico

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The population structure description and analysis of the cycad *Dioon purpusii* Rose were carried out in three sites located in the state of Oaxaca, Mexico. Phenological and dasometric variables of 737 individuals, plant density and covering were analyzed, as well as environmental and floristic variables. Seedlings, juvenile and adult plants, both male and female, were present in the three sites. The analysis of variance revealed that significant statistical differences exist (Duncan test $p < 0.05$) among sites only for stem diameter and height. Plant density and covering was higher in site 1 than in the other two sites, which could be explained because of xeric characteristics in this site, where other species of the tropical dry forest are not able to survive excepting cacti. The similarity analysis including the phenological, dasometric, environmental and floristic variables of the three sites, denoted that sites 1 and 2 were 97% similar, and site 3 was 84% similar to the other two. This suggests that the three sites represent a fragmented population, although they are 5 km and 0.5 km far one of each other, and it is threatened.

P2457. The 2002 flood impact on the aquatic vegetation in selected parts of the Austrian reach of the Danube River

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The aquatic vegetation of the Austrian reach of the Danube River was first surveyed in 1995. Selected parts were re-examined in 2004, in the second vegetation period following a catastrophic flood in 2002, and respective changes were recorded. Three selected reaches are parts of the impoundments of hydro-electric power plants in Abwinden-Asten, Wallsee-Mitterkirchen, and Greifenstein, and one reach is situated in the non-regulated Wachau valley. The Distribution Diagram and the Mean Abundance (MMO/T), a graph and a metric describing aquatic vegetation distribution types are in common use in all countries along the Danube River. They are used here to describe the changes in macrophyte occurrence and abundance between 1995 and 2004. In general the impact of the 2002 flood is clearly shown in river parts where plant species susceptible to water flow were found. Even mosses were affected by this great flood. Modifications in distribution type are discussed in detail for some aquatic species which are important members of the macrophyte community in the Austrian reach of the Danube.

P2458. Fractal patterns in species distributions of some British scarce plants

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The spatial distribution and fractal structure of two British scarce plants, *Lobelia urens* (heath lobelia) and *Phyteuma orbiculare* (round-headed rampion), have been examined at several different scales.

The two species have similar degrees of local patchiness at scale coarser than 50 km and have contrasting coarse-scale between 50 km and 1 km scales, but differed consistently in the slopes of their scale-occupancy curves distributions at scale finer than 1 km. The slope of the log-log plot of *L. urens* is not constant, but varies

systematically with **spatial** scale, and from habitat to habitat at the same **spatial** scale.

Abundance estimates suggest that the species *P. orbiculare* is found to be clumped at all scales, whereas *L. urens* is dispersed at intermediate scale. Fractal dimension analysis suggests that this changes through scale. The distribution varied in their pattern from highly clumped to randomly dispersed. Fairly predictions of *L. urens* can be made from 50 m and 200 m.

Some issues affecting management of species abundance, as well as underlying mechanisms and conservation schemes have been highlighted.

P2459. Fractal patterns in species distributions of some British scarce plants

G. Chekuimo¹, W. Kunin², M. Pocock², R. Aston²;

¹TRAFAM, Yaounde, Cameroon, ²Faculty of Biological Sciences, School of Biology, Centre for Biodiversity and Conservation, Leeds, United Kingdom.

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P2460. Pollination disturbance by alien species in Ogasawara Islands.

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Pollination network in the oceanic island, Ogasawara, was heavily disturbed by alien lizard and introduced honeybee. Human inhabited Chichi-jima and Haha-jima Island had less diverse visitor fauna than in uninhabited satellite islands. Introduced honeybee highly dominated for flower visitation rate in Chichi-jima and Haha-jima. Especially, the alien flowers were visited more frequently mainly by honeybee in these islands but less frequently than the native flowers in satellite islands where the native pollinator fauna was preserved. Thus, the alien plants could overcome one of barriers for naturalization at least on pollination stage in Chichi-jima and Haha-jima.

Food experiment showed that alien anole lizard was aggressive predator for native visitors. In addition, the distribution among islands and introduced history of the anole suggested that this predation pressure was the main reason of native pollinator decline. These two aliens may not only disturb the plant reproduction but also shift the plant-pollinator co-evolutionary direction through the pollination disturbance.

P2461. Herbivory and Hidrodinamism: two important factors determining the community structure on a rodolith bed in southern Brazil.

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Federal University of Santa Catarina, Florianopolis, Brazil.

The role of herbivory on the distribution and abundance of epiphytobenthic community associated to a rodolith bed in the Marine Biological Reserve of Arvoredo Island, Santa Catarina, Brazil, was evaluated through herbivory exclusion experiments. Cages fully and partially covered by 1cm mesh and uncovered cages were randomly distributed at 7 and 13 meters depth on the rodolith bed for approximately 2 months. The results showed a decrease of epiphytobenthic biomass inside total and partial enclosures as compared to open control plots as well as a large heterogeneity. The increase of herbivory by mesograzers inside the cages, evading predators themselves, is suggested as a hypothesis to explain the results. However, the hidrodinamism, as

the herbivory, seems to be another important conditioning factor on the studied rodolith bed. The large heterogeneity of this community points to the need to consider this premise during further ecological experiments in this environment.

P2462. The study about the conditions of the sprout of *Eurya leferox* which is in the crisis of the extinction for teaching materials

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Euryale ferox, the only species in the genus, is a very large water lily covered with numerous spines on all surfaces. *Euryale ferox* used to be a harmful plant for farmers who cultivating rice field in Japan. This plant is considered to be relic species and in a fix of the extinction in some areas of Asia as well as Japan.

They considered it is difficult to cultivate this species. But I try to cultivate this species for teaching materials. I collected wild *Euryale ferox* at Saga city, south part of Japan in August-September 2003. After 2 months of cultivation, I succeed to get 549 pieces of seeds. By using those seeds, I tried to study the conditions of sprout of *Euryale ferox*. Seeds need to store in water to prevent sprout.

After cold processing (12C 30 days) and removing the operculum, 13.3 % seeds of *Euryale ferox* sprout at room temperature and higher (20-26C).

On the other hand, without cold processing no seeds could not sprout.

With cold processing, without removing the operculum 10 % seeds sprouted.

My experiment revealed it is not impossible for school teachers and students to use this plant for teaching materials.

P2463. Seedling morphology and anatomy of *Agave marmorata* Roez. an endemic species from the Valley of Zapotitlán, Puebla, México.

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The Agavaceae family has anatomic and structural characters enabling these plants to adapt to different environmental conditions, mainly to arid zones. Little is known on seedling characteristics that could correlate with their natural environment. The anatomical and morphological features that enable *Agave marmorata* seedlings to survive under an extreme environment were obtained. Seeds were germinated on perlite or a 1:1:1 sand, perlite and soil mixture, for 16 months. Seedlings structural changes were registered and anatomical description was done. Young seedlings of *Agave*, develop linear leaves from cotyledon leaf in a levogirous rosette morphology. Their anatomical arrangement is symmetric, the parenchyma cells have a big central vacuola, lined up sunken stomata with stomatal cavity, a wax covered epidermis whose thickness depends on the substrata where it grows. All these characteristics enable *Agave marmorata* seedlings to establish under this harsh arid environment

P2464. Entomofauna associated to *Agave marmorata* Roez. during its flowering period.

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Agave marmorata Roez. is an endemic perennial species from the Mexican states of Oaxaca-Puebla. It is a semelparous plant that develops its inflorescence during the months of April-June. Flowers produce considerable amount of nectar over the day which attract many visitors. Diurnal floral insect visitors were registered for this species. We selected hazardously eight flowering agaves available in the study area. Observations and collection of insects were performed from 6 am to 6 pm. All insects were collected by different methods: direct collection on the inflorescence, leaves and flowers; the use of insect net and the use of aspirators. A total of 360 specimens were captured and classified in five orders: Hymenoptera, Thysanoptera, Coleoptera, Diptera and Hemiptera. Pollination has been considered possible

for hummingbirds and bats; instead insects were categorized as nectar robbers; here we suggest their possibility as pollinators due to their behaviour.

P2465. Conservation of Ecosystem: Role of Sacred Groves in India.

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The word sacred has been interpreted not in a narrow sence of religious belief alone but to cover all places which people have chosen to care for whatever be the reasons. The sacred goves(SG) are rich in biological diversity and natural ecosystems, some of which however may have aquatic features like waterfalls and subterranean water flows. Himalayan region, Eastern and Western Ghats are hotspots of India in which no. of SG with high degree of endemism and ecological gene pools. People of old generations chose many important medicinal trees for veneration and worship. A temple with water tank and some important medicinal plants is one of the basic things in villages of India. This is the method of water harvesting and In situ conservation of ecological and economic keystone species. Each SG is rich in its history which sustain and support life under a given agro-ecological condition. Unfortunately many of these SG disappearing under the influence of modernisation and ruthless exploitation of these resources to meet the industrial needs. Measures should be taken to protect and restore such of them which had lost some of their pristine glory of their earliest state

P2466. The coriander collection in the German ex situ-genebank in Gatersleben - state of conservation and evaluation

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Coriander (*Coriandrum sativum* L., Apiaceae) is a major spice plant of the Indian sub-continent. Leaves, seeds, and essential oils are used as spice material. The species has a wide range of distribution and a formal taxonomic distinction of three subspecies with ten botanical varieties. The coriander collection of the German genebank in Gatersleben covers 457 accessions from more than 60 different areas of origin. All subspecies and varieties are available. A complete morphological description of the different leaf shapes, the colour of the flowers, the size and shape of the fruits, and the thousand grain weight is required to characterize the accessions and to determine the botanical classification. Despite optimal storage conditions (-15°C, moisture content <10%), a natural seed ageing process accompanies long-term storage in a seed bank. In order to find the longevity of the seeds a control of the germination rate is necessary at regular intervals. Genebank material should be used for scientific research also. 380 different accessions are in study to evaluate the winter hardiness and the survivability under strong environmental conditions.

P2467. Begonia Germplasm Resources of China

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One hundred and seventy-two taxa of *Begonia* L. are known in China. They are distributed in twenty-one provinces, regions or districts in China. One hundred and six known taxa are distributed in Yunnan, which occupies nearly 62% of the total Chinese begonias. The lowest elevation of the natural distribution of the Chinese begonias is 90m and the highest elevation is 3400m. Among 172 taxa of the Chinese begonias, 93 taxa belong to rhizomatous type, 42 taxa belong to erect-stemmed type and 37 taxa are tuberous begonias. It was noticed that over 50% of the Chinese begonias have ornamental value. Many Chinese begonias can be directly developed as ornamental plants. Some Chinese begonias are very precious germplasm for breeding purpose. Some species like *B. sillebensis* have scented flowers. The yellow-flowered begonias are also valuable germplasm resources for breeding purpose. Many Chinese begonias are naturally distributed in a very small area and some begonias are threatened for surviving. Hence, the efficiency conservation of the Chinese Begonias gets no time to delay.

P2468. The Role of Ex Situ Institutions in Plant Conservation: Measuring Success.

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The Global Strategy for Plant Conservation (CBD, 2002) and other international policy documents have provided conservation organizations with a framework and targets for conserving plant diversity. Similarly, funding agencies generally request or require a grant recipient to demonstrate what the funding has accomplished. Botanic gardens and other *ex situ* organizations are increasingly faced with the challenge of measuring and articulating the success of their applied conservation programs. Perhaps the most appropriate yardstick for assessing success of conservation projects is, "Does it directly contribute to conserving the resource (e.g. plant diversity)?" Success can be measured in numerous ways including resources conserved, improved management implemented, better laws and policies, changes in knowledge, attitudes and behaviors of stakeholders, and reduction of threats to biodiversity. We discuss the results of a survey on botanic garden conservation programs, the benefits of measuring a garden's conservation success and several tangible and quantifiable performance indicators.

P2469. The Macea University Botanical Garden Collection of Quercus I. Genus

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Member of the Botanical Gardens Association of Romania, the University Botanical Garden of Macea, 21.5 hectares, represents a scientific collection of 2,150 wood taxons, about 1,100 herbal species.

Nowadays, consists of 42 taxons, 35 species, one variety and 6 cultivars. Among these, 10 are indigenous taxons, the rest of them are exotic oaks.

The 7 monumental examples of *Quercus robur* L. of the Garden, that are between 350 and 400 years old, are well vegetating, without showing any traces of physiological regress. Due to their advanced age, there are three monumental examples of *Quercus rubra* L.: *Q. macranthera* Fisch. et Mey and *Q. petraea* (Mattuschka) Liebl. '*Mespilifolia*', cited in the speciality literature, that are lost.

Rarities of European dendroflora: *Quercus bucklet* Micon et D. Don, *Q. fabri* Hance, *Q. gambellii* Nutt., *Q. muehlenbergii* Engelm., *Q. nigra* L., *Q. shumardii* Buckl., *Q. texana* Buckl etc.

. Sectors that are intensely visited by public present oaks of ornamental value, such as *Quercus coccinea* Muenchh., *Q. shumardii* Buckl., *Q. texana* Buckl. There are also *Q. macrocarpa* Michx with a special rhytidome, *Q. robur* L. *Fastigata* and *Pendula*"

P2470. Conservation and Utilization of Plant Genetic Resources in Nepal

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The plant biodiversity of Nepal consists: cereals, grain legumes, vegetables, industrial crops, oilseeds, spices, fruits, ornamentals and herbs. The contribution of plant biodiversity to food supply, agriculture, forestry, medicine and industry is enormous. Nearly 54 percent of Nepal's physical landscape is covered with vegetation (37 % forested area, 5 % shrub land and 12 % grass land). A total of 118 ecosystems with 75 vegetation and 35 forest types have been identified in Nepal. Though Nepal possesses only 0.1% of the total landmass of the world, it harbors nearly 2.5 % flowering plants, 3% pteridophytes and 6 % bryophytes of the world's flora. There is high degree of endemism (246 species), which accounts for 30 % for whole of the Himalayan region. There are more than 6,500 plant species of which more than 500 plant species are edible, 700 species of medicinal plants have been identified and a total of 10,736 accessions of 90 crops have been documented. Nepal is considered to be one of the countries of origin of rice in the South Asia. Existence of more than 120 species of wild relatives of cultivated crops is an additional dimension to the plant genetic resources of Nepal.

P2472. Impact of Afghan Refugees on Flora of Pakistan - A Case Study: Kurram Agency

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The research area, Koh - e - Sufaid Range, lies between 33°-53' and 34°-03' north latitudes and 69°-50' and 70°-51' east longitudes, in Kurram Agency, Pakistan.

Traded fungi and medicinal plants of the area were *Morchella esculenta*, *Artemisia brevifolia* and *Teucrium stocksianum*. Their estimated annual consumption was 240,200 kg. Annually, 114,000 Kg of *Artemisia brevifolia* and *Teucrium stocksianum* were also smuggled from Afghanistan to Pakistan. The local people reported that, in Afghanistan, *Artemisia brevifolia* is found insufficiently as compared to *Teucrium stocksianum*. *Artemisia* of Afghanistan is of red color while that of Kurram is of green color. *Teucrium* of Afghanistan is larger than Kurram. About four trucks of *Teucrium stocksianum* while three of *Artemisia brevifolia* were brought from Afghanistan by Jagi tribe and sell to the mentioned trader. Majority collectors of these two species were men. Data analysis shows that about 64,000 kg of *Teucrium stocksianum*, while 50,000 kg of *Artemisia brevifolia* were carried in Pakistan through that route.

P2473. Enrichment of dendroflora biodiversity in Belgrade region with some alochtonous species

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Belgrade region is phytocenologically determinate by *Quercetum frainetto cerris*, with characteristic, mostly deciduous dendroflora species. The possibility of introducing new, ecologically suitable, especially species resistant on phytopathological diseases, insects and increasing air and soil contamination, is extraordinary valuable. Introduction of coniferous is important from the different points of view. Besides the enrichment of biodiversity, the esthetic values of landscape increases and the new ambient with healthier conditions is more suitable for recreational activities. The paper presents the researching results of three very interesting alochtonous species adaptability and development on the several locations. Besides the quantitative parameters, the variability of analyzed characteristics has been presented by statistical parameters. The results confirms that these species has very intensive increment, there has not been identified any kind of harmful insects or phytopathological diseases, and the esthetic values are significant.

Key words: Belgrade region, *Cedrus atlantica glauca*, *Taxodium distichum*, *Quercus borealis*.

P2474. Dependency of biological impacts of fixed part (GSM) equivalence on frequency

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Considering potential electromagnetic environmental stress there is a physical, political and economic need to investigate its impacts on living beings and its consequences on environment and human society in general. For that purpose the Environmental Department of ARC Seibersdorf research GmbH developed and built a new experimental set-up for research of biological impacts of high frequency electromagnetic fields on plants. 17 chlorophyll fluorescence (CF) and 13 standardized growth parameters of red radish seedlings (*Raphanus sativus* L.) were evaluated. Univariate ANOVA tests showed significant main effects and group differences between some of the independent variables.

Here we discuss the evaluated data not only as implication of field-strength (classic viewpoint) but also as composed effect, where the field-frequency is presented as one relevant factor of the biological impacts of high frequency electromagnetic fields on plants.

Another goal of our research is that some of measured CF-evaluation parameters (like F0, F1, F2) clearly show their forecasting potential.

P2475. Ex situ plant conservation in Greece: The case of the Balkan Botanic Garden of Kroussia**E. Maloupa;**

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Conservation of the Greek and Balkan native plants involves a significant part of the research and activities taken place in the Balkan Botanic Garden of Kroussia (BBGK). Living plant material has been carefully collected from nature, accompanied with all necessary climatic, soil, location and individual species-properties, in order to withstand the treatments applied for conservation in the ex situ environment of the nursery and BBGK. Plant conservation efforts produced 855 native and floricultural species, which have been taxonomically identified and preserved ex situ in BBGK.

Despite the floristic richness and high endemism, the establishment of more new Botanic Gardens and network in Greece is limited. However, initiatives have been undertaken by BBGK at national level and moreover with neighboring Balkan. There is no previous experience and information on how to cultivate almost all of the species preserved ex situ in BBGK, therefore, efforts for the development of new propagation protocols found to be necessary, especially for 263 threatened Greek endemic taxa.

P2476. Seasonal variations in moisture, ash, and lipid content of three edible seaweeds from the Chincoteague Bay, VA, USA**M. Mitra, J. G. Schwarz, I. Khan;**

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Of all the bays in the Delmarva Peninsula, the southern Chincoteague Bay, Virginia, has the greatest richness of edible seaweeds. Although these seaweeds have the potential for use as a food source, few studies have examined seasonal changes in moisture, ash, and other nutritional composition in macroalgae. A preliminary study was undertaken to detect seasonal changes in some of the proximate constituents such as moisture, ash, and lipid of three abundant species of edible seaweeds from the Chincoteague Bay. The three species, *Gracilaria tikvahiae* (Rhodophyta), *Fucus vesiculosus* (Phaeophyta), and *Ulva lactuca* (Chlorophyta) were sampled in summer-fall and winter-spring during 2004-2005 and analyzed for moisture, ash, and fat. Results show that moisture content (%) was higher in all the three species during summer-fall than winter-spring. Ash content (% of dry weight) was lower in spring for all species. Lipid level was elevated in *Fucus* during the winter but fluctuated less in *Gracilaria* and *Ulva*. These seaweeds are exposed to seasonal variations of abiotic factors that could influence their metabolic and growth responses, and also impact herbivory.

P2477. The Effect of war on Agro-biodiversity In Sierra Leone**R. T. M. Chakanda;**

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Sierra Leone is recovering from a ten-year long civil war that devastated the patterns of livelihood of the farming communities that uphold agro-biodiversity. There is a need for information about the status of, and the impact of the war on agricultural biodiversity. The way this knowledge is gathered encompasses:

- 1) a survey in the post-conflict situation to assess the left over of on-farm diversity. This is highly dependent on farmer knowledge much of which is subject to erosion over extended periods.
- 2) pre- and post-war comparison of agro-biodiversity. Since there are virtually no undisturbed locations within Sierra Leone and there are hardly any records to outline pre-war and wartime crops diversity regimes. For this, a comparison is made with the border region of neighbouring Guinea.
- 3) assessment of the existing level of diversity in a number of crops based on morphological observations on the collected accessions, involving field trials and subsequent molecular characterization.

P2478. Modelling plant species richness on a regional scale in a man-dominated environment**J. Wahr¹, S. Klotz^{1,2}, T. Wohlgemuth³, V. Mosbrugger¹;**¹Institute for Geosciences, Tübingen, Germany, ²Laboratoire PaleoEnvironnements et PaleobioSphere Université Cl.Bernard, Lyon,France, ³Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland.

Analytic studies on regional biodiversity patterns are still rare. The present study aims to reveal the potential factors of influence on the spatial biodiversity patterns (species richness) of all vascular plant species and selected species groups (native, alien, red-list) on a regional scale in a man-dominated environment (SW-Germany, Baden-Württemberg).

Floristic data rely on a regional floristic mapping scheme with a grain of 5.5*6 km per grid cell and an extent of 931 grid cells covering an area of 30,000 km². Out of available environmental data (climate, geology, soils, landuse, etc.) about 200 parameters are processed using GIS. To predict regional plant species richness according to environmental variables multiple linear and non-linear regression models are applied.

The results suggest that multiple linear models may explain about 40% of the variability in the total number of all plants, and up to 60% of the variability in the total species-number of specific plant-groups. Non-linear models could slightly enhance the quality of the prediction. A rough assessment of biodiversity change on the basis of land-use and climate change scenarios is done.

P2479. More humans means more plant species in the Eastern Caribbean**L. E. Chinnery;**

The University of the West Indies, Bridgetown, Barbados.

Expected relationships were found for species number and area and altitude for pteridophytes in the Eastern Caribbean. However, the slope of the species-area relationship ($z = 0.756$) was greater than expected (~ 0.301) and there was a positive correlation between human population density and species number. An explanation is that with increased population there is likely to be at least one person interested in ferns and involved in activities to increase knowledge of an island's flora. If true and if the number of such individuals increases with population size, this would induce a positive correlation and contribute to the increase in the species-area slope. Alternately, the existence of unequal sampling intensities with the floras of the large islands being better known than those of the small ones would contribute to an increased value of z . Data of other groups from the 'Flora of the Lesser Antilles' were used to see if the species number/human population density relationship applies to other vascular plants or which sub-groups it applies to. For groups tested, z values were greater than expected and there were positive correlations with human population density.

P2480. Multiple-use native trees: a biodiversity recovery tool.**M. Equihua, G. Benitez, M. Pulido-Salas;**
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In Veracruz, Mexico problems and issues associated with markets in meat, coffee and sugar cane could spur changes in land use. The situation opens-up an opportunity to recover biodiversity by afforestation using native species. In Veracruz there are some 950 species of trees, which represent 43% of the 2200 tree species estimated for all of Mexico. In this study we report on 107 species that are propagated in official tree nurseries in Veracruz. The study combined a taxonomic description of these species with the review of ecological and horticultural data. Highlighted in our results are uses that seek to find viable production alternatives that also serve a conservation function. Multiuse species were considered in both agroforestry and silvicultural/pasture system. We also report on three application oriented toward increasing knowledge on species from tropical montane cloud forest, one of the most threatened communities in Mexico: a program of restoration with 11 tree species from the cloud forest, an evaluation of the meaning of contrasting conservation status of *Carpinus caroliniana*, a study of the spatial distribution and protection of *Ostrya virginiana*.

P2481. The effect of plant diversity on people's perception and appreciation of vegetation patches**P. Lindemann-Matthies¹, X. Junge¹, D. Matthies²;**¹University of Zurich, Zurich, Switzerland, ²University of Marburg, Marburg, Germany.

To study people's perception and appreciation of plant diversity, we arranged (1) plants in meadow-like arrays of different diversity (1, 2, 4, 8, 16, 32, 64 species) and (2) marked vegetation patches of different levels of plant diversity along waysides. We presented the plots to more than 1000 people and recorded their estimation

of plant diversity, their preferences for certain plots, and their botanical knowledge. Study participants were generally able to distinguish between different levels of diversity. However, they overestimated the diversity in the low diversity plots and underestimated the diversity of the high diversity plots. The number of species perceived increased linearly with log species number, i.e. the participants perceived each doubling of the number of species as a constant increase. People's appreciation of a plot increased continuously with species diversity. The results indicate that species-rich plant communities are appreciated, which is an important condition for their protection. However, people perceive plant diversity inadequately and may thus not notice the ongoing decline in biodiversity.

P2482. Conservation of biodiversity anthropocentrism vs biocentrism *in situ* and *ex situ* paradigm shift

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Biocentric consciousness and ecological awareness is a result of continuous environmental depletion caused by prevailing non-sustainable conditions created by anthropocentric activities of human society. Transformation and modification of natural assets, domestication of faunal and floral species, modernization of physical landscape, urbanization, industrialization, world wide network of means of communication and transportation have fasten the rate of environmental degradation. The biodiversity is under threat of extinction and erosion. Rapidly increasing number of endangered species is a threat to humankind for his survival and sustainability. Non-sustainable and monistic approach of anthropocentrism must be replaced with sustainable and holistic biocentric approach so as to maintain and protect biodiversity including man himself.

Attempt has been made to understand and explain *in situ* and *ex situ* paradigm shift in terms of biodiversity conservation taking into consideration biocentric holistic approach in place of anthropocentric monistic approach with the help of poster presentation.

P2483. Emergency situations and Life Support Species

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Hazards and disasters like Tsunami have given a wake up call to human race and new awareness that science and technology needs to do more for vulnerable communities, disappearing habitats and vanishing biodiversity. Precious lives are lost along with unique cultures who depend on prevailing precious biodiversity. New awareness in science and technology of biodiversity is required to meet the needs of traditional communities. Life Support Species (LSS) are locally adapted unique plant, animal and microbial genetic resources which have specialised biological attributes to withstand extreme environments and are used for survival and food, nutrition, energy and environment needs by local cultures and communities. The ancient civilisations derived their knowledge from the signals provided by local plant and animal diversity of LSS to take precautions and avoid risks to life from impending disasters. The gene pool diversity of these species can meet future needs emerging due to extreme environment changes. Example of Chenopods and other such species are discussed. A Global Action Plan on LSS is recommended.

P2484. Digital mapping of fruit trees as an instrument to monitor diseases and conserve varieties

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A computer supported method was tested to map fruit trees and host plants of fire blight. Fire blight is caused by *Erwinia amylovora* and is an infectious bacterial disease, which can attack very dangerously many Rosaceae, e. g. cultivars of apple (*Malus communis*) and pear (*Pyrus communis*). The practical work was done during the summer 2003 in Fußach, Vorarlberg, where 3463 trees on an area of 675 ha were mapped. Most of the fruit trees grow in extensive orchards ("Streuoobstanbau") where lots of different and old fruit varieties can still be found. Aerial photographs saved on the pocket computer helped to orientate in the field and determine the exact position of the plants to map. The special programming of the GIS-application used made it possible

to register all necessary data immediately by typing on the touch screen of the pocket computer. The result is a digital tree map, which makes the spreading of fire blight visible and allows an easy update of newly infected plants in following years. Thus, unharmed trees, which could be tolerant or resistant to the disease, can be found very easily. The method can also be useful to map fruit trees for plant conservation aims.

P2485. Ecological Factors Affecting Iranian Annual Medics

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Ecogeography of *Medicago* species was studied in Iran where is considered as one of the primary centers of diversity for this crop. Annual medics collected from across the country were characterized, indicating that 21 annual *Medicago* species are grown in naturally different ecological zones. Results showed annual medics were distributed mostly in the North-West, West and South of Iran. The relationship between geographical diversity and ecological factors was studied showing that altitude and precipitation were the two important factors in the distribution of *Medicago* species. Most of the species distributed from 0-2750m above the sea levels in areas with 100-400mm annual rainfall and the Mediterranean climate. Soil texture analysis showed that annual medics are generally grown in Loam or Clay soils with pH=7.0-8.0. However, some species were found in ecologically marginal and different areas. Among these species *M. rigidula* and *M. rigiduloides* are adapted to cold zones, while *M. laciniata* and *M. sauvegei* were found in Southern Iran with the annual mean temperature of 20° C. *M. littoralis* is a littoral plant which grows near the Caspian Sea and the Persian Gulf.

P2486. Origin of waxy foxtail millet

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Naturally occurring waxy and low amylose variants of foxtail millet (*Setaria italica*) arose in a limited region such as East and Southeast Asia as other cereals under human selection for sticky foods. We determined the structure of *GBSS1* gene conferring these traits by genomic and RT- and RACE- PCR, and analysed structural differences among 871 accessions with these three phenotypes by PCR. As a result we found that these traits were originated by independent insertions of transposable elements and subsequent insertions into these elements or deletion of a part of the elements. The structural analyses of transposable elements inserted into the *GBSS1* gene revealed that changes occurred from non-waxy to low amylose once, from non-waxy to waxy three times, from low amylose to waxy once and from waxy to low amylose once. The present results and the distinct geographical distribution of different waxy molecular types strongly suggested these types originated independently and dispersed into the current distribution areas. Foxtail millet can be a new model for transposable elements- mediated evolution under human selection.

P2487. Variability of economic traits in the old varieties population of opium poppy (*Papaver somniferum* L.) with the Slovak origin

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Opium poppy (*Papaver somniferum* L.) belongs among traditional species cultivated in the Slovak regions for many centuries. People from some Slovak regions are still cultivating old and marginal species. Collecting and reproduction of old and landraces and evaluation of their economical traits were ensured. 55 genotypes were experimentally tested. In the traits level was determined range - plant height 1102.7 - 1514.0 mm, number of capsules 1 - 9, capsules length 10.9 - 93 mm, capsules width 8 - 59.4 mm, capsules weight 1 - 29 g, weight of seeds in capsule 5.1 - 13.7 g. Significant differences were determined also in the shape and the size of the capsule, flowers color and in other traits. Experimental data were processed in the information system GENOTYPDATA Papaver. Information system provides characterisation of the genotypes according to unified classifier also with the image documentation.

P2488. An investigation of the inheritance procedure and estimating the number of controlling genes of some traits related to grain yield in barley (*Hordeum vulgare*) Afzal/Radical cross

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In order to study inheritance of traits related to grain yield in barley, F₁, F₂ and F₃ generations were made from Afzal/Radical cross. Parents along with F₁, F₂ and F₃ generations were planted in a randomized complete block, design with three replications. Head weight, head length, number of heads, number of spikelets per spike, awn length, hundred grain weight, grains per head and straw yield per head in different generations were recorded. Analysis of variance indicated that mean squares of generations were statistically significant for all the traits. Then, generation mean analysis was performed for all the traits. The results showed that additive, dominance and epistasis effects were effective for all the traits except awn length. Recently trait was controlled with only additive and dominance effects. Also, results indicated that dominance variance was the most important for inheritance of all the traits. Average broadsense heritabilities were between 69-89% for all the traits. The number of genes for the traits were estimated to be between one and five.

P2489. Study of saffron plant (*Crocus sativus* L.) adaptability in North West of Iran

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Iran is major country of high quality saffron production in the world. Traditionally the crop is produced in arid region of east of Iran (Khorasan province). An experiment was conducted to study of adaptability of the crop in Ardabil province in North West region of Iran which has cool summer and hard winter during 2002 to 2004 years. Three important ecotypes of saffron from east of Iran including, Torbat, Kashmar and Ghaen were selected and cultivated their corms in Ardebil province. Dry matter weight and length of stigma, mean yield/ha and mean yield total/ha for each ecotype were measured. The results showed that Kashmar ecotype with mean yield total/ha of 5.77 Kg had significantly higher production in compare with two others. Mean yield total/ha of Torbat and Ghaen ecotypes was 4.71 and 4.62 Kg respectively. Mean yield total/ha of the three ecotypes with 5.03 Kg was greater than their production in native region which is 3-4 Kg. Dry matter weight and length of each stigma were 0.0062 gr and 37 mm respectively. With respect to mean yield total the crop has good compatibility and tolerance to Ardabil cool and hard weather; therefore it may be profitable crop in the region.

P2490. Researches regarding the dynamics of the respiratory process in rye plants parasitized through artificial inoculation with various peptide alkaloid strains of *Claviceps purpurea* (Fr.) Tul.

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The intensity of the foliar respiration at the parasitized plants depends on several factors. In the same circumstances, under the same environmental conditions, different species have different respiratory intensities. The respiratory process in rye plants was observed from the beginning until the end of the growth period of rye and of *Claviceps purpurea* parasite as well. During this period the foliar respiration presents high variations from one stage to another. The presence of the parasite determines important changes related to the predominant alkaloid type of the *Claviceps purpurea* strains, which parasite the rye. The intensity of the foliar respiratory process within the pathogen system rye -*Claviceps purpurea* is evidently influenced by the ontogenetic state of the host, by the infecting alkaloid type strains, as well as by the environmental conditions host plants are grown in, which allow a certain degree of infection in the individuals affected by the disease.

P2491. Sulfur-oxidizing rhizobacteria and *Bradyrhizobium* association for better soybean production

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Present investigation was carried out to develop an inoculant product using a synergistic association between a naturally occurring sulfur (S)-oxidizing plant growth promoting rhizobacteria (PGPR) and a nitrogen (N)-fixing *Bradyrhizobium* that would enhance the soybean growth. Soybean (*Glycine max* L.) is a major field crop in North America especially in US and Canada. Farmers in the region are familiar with the using of N-fixing inoculant for proper root nodulation resulting in greater N supply to plants. The use S-oxidizing rhizobacteria *Delftia acidivorans* and *Bradyrhizobium japonicum* association showed improved soybean nodulation and vigorous plant growth. Results from our multi site field experiment showed that with the increased nodule number in plants soybean yield was also increased significantly with this unique microbial product. This product combines a S-oxidizer and a N-fixer, which may be the first to initiate a commercial development of a new consortium based inoculant for better soybean production.

P2492. Diversity of *Musa Balbisiana* Colla and its AAB/ABB hybrids in Thailand: Insights from AFLPs

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It has been speculated that Thai AAB and ABB banana may not have originated in Thailand; rather these might have been introduced from Malesia into Thailand long time ago. However, recent molecular study suggested that these Thai banana hybrids may have different origins. In order to clarify this argument, we conduct an investigation using AFLP markers in accessions from *Musa balbisiana* Colla and its AAB- and ABB-hybrids collected in Thailand to access their diversity and genetic relatedness. Accessions from neighboring countries were included as the reference in analyses. The results shown that ABB accessions were closely related genetically; so did AAB accessions. With respect to Thai accessions, it was suggested that there might be two origins of AAB/ABB hybrids: one possibly occurred in Thailand and the other was in the Pacific islands. These results certainly provided implication on domestication of these hybrids in Thailand.

P2493. Genetic and environmental interaction in Uzbekistan walnut nuts

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Walnut is extended from south Europe to Central Asia. The wild forests in Uzbekistan are in western Tien-Shien and Pamir-Alay from 800 up to 2300 m. Walnuts are under the influence of anthropogenic factors and reduction of the forest goes to biodiversity erosion. Biochemical characterization of nuts from plus trees is a step to evaluate the biodiversity and environment interaction. Size and weight give a preliminary indication of biodiversity. The oil, protein and vitamin E are analyzed. Nuts from mountain trees have higher water content and some samples have higher oil percentage. This observation can be related to genetic background more than the environment because some samples from dry area have high oil percentage. From fatty acids analyses nuts with high linolenic/linoleic ratio are identified. Linolenic acid is important for oil quality. Two other fatty compounds are in oils from only one area. Also vitamin E changes in relation to the different samples analyzed. The comprehensive of the data indicate wide biodiversity and environmental action. The research will be completed to evaluate and distinguish between genetic background and environment.

P2494. Collecting Portuguese maize landraces with technological ability for bread production

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Maize was introduced in Portugal on the XVI century. Since then, natural and human selection lead to a very diverse germplasm, recently confirmed by SSRs (Vaz Patto et al., 2004, Euphytica 137:63-72). Most Portuguese maize landraces are white flint types appropriate for bread production, representing valuable source of interesting genes. The traditional maize bread (broa) still plays an important economic and social role on rural communities, characterized by small farms relying on polycrop, quality oriented, sustainable systems. However, these are in risk of disappearing because of the progressive adoption of intensive hybrid varieties not suitable for bread production.

Expeditions were made to collect maize landraces among traditional farmers. The cultural practices and constrains, soil type, ethnobotanic parameters and associated crops were also collected (50 maize landraces, 120 varieties of different legumes and rye). We will analyse their technological ability for bread production, valorising them and promote on farm conservation and participatory breeding programs on the rural communities, contributing to a sustainable rural development.

P2495. The studies of biological peculiarities of *Primula malacoides* Franch. Varieties and hybrids

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Primula malacoides Franch. has been cultivated in Kaunas Botanical Garden, Vytautas Magnus University for a long time, its selection started in 1946. Five new original, plentiful of flowers, high ornamental quality, and scent varieties - has been developed and legalized in 1967-1976.

New hybrids were created in 1995, perspective candidates to the new varieties - *Žydre*, *Vakare*, *Rubinas* and *Margute*. First indication data about new-created hybrids were published in 1999 to 2001 these hybrids have been registered in Lithuanian register of flower varieties.

Aim of research - to create new hybrids of *Primula malacoides* Franch., to investigate and compare biological (morphological, genetical and physiological) peculiarities of four earlier-created varieties and new-created hybrids, evaluating plants ornamental quality during the period of growing and development.

P2496. Indigenous traditional knowledge integration for forest biodiversity conservation in India

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Indigenous traditional knowledge (ITK) on the flora of India is as old as ancient scriptures, bio-geographical niche, cultural history and natural resource. Indigenous communities represent nearly 430 distinct ethnic groups interspersed among 54 million tribal people under 227 linguistic groups and inhabit different phytogeographical locations. The wild resources are the result of co-evolutionary relationships between indigenous peoples and nature since time immemorial. Ethnobiological information on nearly 10,000 wild plant species including 7500 species for medicinal purpose, 3900 subsidiary food, 525 fibre and cordage, 400 as fodder, 200 pesticides have been documented. The products/outputs and services through the integrated ITK approach proposed are: characterization of ethnobiological significant areas, registration of grassroots innovations, taxonomic characterization; reintroduction of threatened species, preparation of extension materials, gender initiatives, benefit sharing, developing at organizational and local government level, accessibility to IK rich areas, and capacity building through integrated approach.

P2497. Preserving biodiversity in the farming system through sustainable management

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Crop diversity is crucial for food production and sustainable agriculture and contributes to food security, nutrition improvement and higher incomes.

In Portugal for each crop there are several landraces that are, frequently, the result of geographic isolation.

Maize (*Zea mays* L.), common bean (*Phaseolus vulgaris* L.) and coles (*Brassica oleracea* L.) are important for food supply. Grapevine (*Vitis vinifera* L.) is used for the production of superior quality wines, contributing for higher incomes.

Maize is used for the manufacture of a special bread "broa", that in the North, plays an important role in rural communities.

Common bean and coles are main constituents of some traditional Portuguese dishes still greatly consumed in the rural communities.

Grapevine autochthone varieties are close to 300 and most of them exist exclusively in the country. Diversity has been preserved not only between varieties but also within the varieties themselves (clonal variability). In order to preserve such diversity growers are encouraged to use a blend of clones in their vineyards and the Portuguese list of varieties for wine making was officially established.

P2498. The state of the art on the archaeobotanical study of Arslantepe-Malatya (Turkey): the role of agriculture and artisanship at the end of the IV millennium B.C.

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Arslantepe, with its millennia of buried history (from Late Chalcolithic to Neo-Hittite age) is an archaeological hill close to the Euphrates river. In the final centuries of the fourth millennium (3300-3000 B.C.) a huge monumental public complex, the first known example of a palace in the Near East, was built at Arslantepe. Only few seeds of legumes, few tens of bad preserved and fragmented wheat caryopses and some grape pips, either of wild or cultivated type, have been found sieving huge sediment quantities. Hundreds of hulled many-rowed barley caryopses were recovered from waste places or corridors and from an open area just outside the gate of the palace. This lack can not be fortuitous and it is plain that the bins were not discovered yet. Preliminary pollen analyses carried out on sediments from the drains dug in one corridor show the presence of cereals pollen and grass cuticles. Phytoliths will be investigated too. Temple B was found with a large amount of big charcoals in situ. The wood used for the big beams of the widest room was mainly of *Alnus*; *Pinus* gr. *sylvestris/montana*, *Fraxinus Populus* and *Crataegus* were used as well, for beams and also likely for smaller items.

P2499. New Aspects on the Diet of the Neolithic Tyrolean Iceman „Ötzi“

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Three surgical interventions on the Tyrolean Iceman conducted in 1995, 1997 and 2000 resulted in the collection of five ingesta samples. These samples constitute a sequence from different locations of the intestinal tract: the ileum, the transverse and descendent colon, as well as the rectum. The samples encompass at least three different meals consumed of the Iceman during his last two days, which is shown by numerical analysis of the pollen flora and muscle fibres incorporated in the different ingesta samples. The macro and pollen analyses of these samples reveal that the Iceman consumed a well balanced omnivore diet. Surprising is the strong correlation between bracken (*Pteridium aquilinum*) spores and human whipworm (*Trichuris trichuria*) eggs as well as wheat (*Triticum*) pollen. This indicates an intentional consumption of bracken as anthelmintic or as starch plant. Additionally the new data corroborate the early season of his demise.

P2500. A survey of storage proteins and enzymes of Iranian diploid wheat seeds

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The wheat is the most field crop in the world and also Iran. It has been investigated from different aspects by now. This investigation led to improve in quality and increase in production noticeably. Among this, diploid wheats are important because of being hexaploid wheats base. Wheat seed proteins including storage and cytosolic proteins are considerable. Our purpose in this study was the evaluation and comparison of enzymes and storage proteins of Iranian diploid wheat (including *Triticum monococcum*, *T. boeotocum* and *T. urartu*). In order to this, we used 25 native masses of diploid wheats usually from the west and the northwest of Iran. In lab, extracts were extracted by proper buffer and then

the total protein and enzymes measured by spectrophotometer. For electrophoresis, SDS-PAGE and A-PAGE were used for gliadin and glutenin respectively and PAGE for enzymes and at last produced bands of these for polymorphism were compared.

P2501. Plants Used in Handicrafts by Indigenous of the Amazon Forest at the Community Monilla Amena (Colombia)

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At the community Monilla Amena, located in the Colombian Amazon, there are at least four indigenous groups (Huitoto, Yucuna, Ocaina and Ticuna) and a mestizo group. We studied useful plants availability, management and spatial distribution. Ethnobotanical information was obtained by interviewing twenty-four adult people. Results yield 77 species used for 71 handmade articles. The most useful families are Arecaceae (25.9%), Moraceae (16.3%) and Aracea (7.6%), and the most useful genera are *Brosimum* (13.7%), *Astrocaryum* (12.9%) and *Heteropsis* (6.7%). One species of *Brosimum* highlights because is used in 23 handicrafts. Most uses involve culture and subsistence activities. Mostly plants are in flood land forest (59.5%), terra firme forest (24.4%) and cultivated zones (13%). We found that 12.8% of plants are cultivated and 87.2% are wild. Huitotos and mestizos reported more species of useful plants (72 and 55), however we did not find significant differences between the numbers of useful plants given by each of the five ethnic groups ($\chi^2 = 0.05$, $p = 0.66$), because there are diverse levels of knowledge between the members.

P2502. Ethnobotanical study of the materials used to build traditional Chontal houses in Tabasco, Mexico

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Chontals have a good knowledge of their resources, that is reflected on the materials used to build their houses since prehispanic times. Today many houses are constructed with bricks and asbestos. Traditions are being lost due to a lack of the materials, caused by human transformation in agricultural activities and cattle raising. The objective was to determine the used materials and to characterize the degree of landscapes modification as well as how this affects the possibility to find resources. Aerial photos were used to delimit the area and characterize landscapes. Surveys were conducted to get information of the materials, where and how they got them and how much they paid to try to evaluate the success of keep on using this type of house. Thirty five species were found in the structure that came from homegardens, agricultural areas or other ecosystems from the north of Tabasco. A lost of tropical forest was found. Therefore there was a lost of materials and a change in some cases to artificial ones. Palm leaves of *Sabal mexicana* are used to build ceilings supported by stems of "jahuacte" *Bactris mexicana*. Both species resist humidity and offer a fresh construction.

P2503. Medicinal Plants Used in Mexico for the Treatment of Rheumatic Diseases.

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The rheumatic diseases are officially recognized as health problem in Mexico. This fact is confirmed by study of traditional medicine herbal resources; a great number of plant species are commonly used to alleviate rheumatic disorders. The objective in this study was to establish the medicinal plants that Mexican people use for the treatment of rheumatic diseases from ethnobotanical information gathered on Herbarium IMSSM. The method applied was the examination of the herbarium vouchers, annotating scientific name, family, common name, and medicinal use. Anthropological and medical criteria were used to organize and to analyze of the information.

We reported 109 medicinal species used for treatment and prevention of 28 popular rheumatic diseases. The more used families are Asteraceae and Solanaceae. The most common used species are *Gymnosperma glutinosum* (Spreng.) Less. (tatalencho); *Persea americana* Mill. (avocado); *Solanum marginatum* L. (sosa); *Urtica dioica* L. (stinging nettle); and *Cannabis sativa* L. (marihuana). Finally, we can conclude that

studies in this area may bring an alternative to the first level of medical care based on herbs.

P2504. Ethnobotanical value and conservation of gallery forests species of Biosphere Reserve of Mare aux Hippopotames, Burkina Faso, West Africa

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The mare of Hippopotames Biosphere reserve is located in the western part of Burkina Faso. It depends on the Province of Houet. It is a part of the humid zone of Burkina Faso. Local populations have a close relationship with the reserve. They get much uses from the gallery forests species; they get much of their needs in food products, medicine, energy, arts and crafts. The use of resources has consequences for the biodiversity, ecosystem and environment of the reserve if the manners are not sustainable. Cutting roots, barks and big stems are not sustainable techniques to the species. The discussions held during ethnobotanical inquiries have permitted us to establish the main use of woody plants and to pick out the repercussion of this usage. From this it emerges that the reserve has an important floristic richness and a biodiversity unknown to both locals and authorities. These two kind of people must work together for a sustainable management of the reserve.

P2505. Medical Ethnobotany of *Pachycereus pecten-aboriginum* (Cactaceae) in Mexico.

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In Mexico, ethnobotanists had paid much attention to some cacti species with the most popular uses, but had been forgotten other cacti with regional importance. The objective of this work is to show the medical importance of *Pachycereus pecten-aboriginum* (Engelm.) Britton & Rose. The method consisted of fieldwork on the Yoreme-Mayo's region, in northwestern Sinaloa state, the record of uses in its natural distribution area, and a revision in IMSSM Herbarium, the main collection of medicinal flora of Mexico, revision of references related with this topic, as well as systematize and to analyze the information. We found that the *Pachycereus pecten-aboriginum* stem and photosynthates ("baba") are used to attend skin illness, traumatism, diseases related to the digestive system, respiratory and urinary system, poisonous animals, diabetes, cancer, and used like enteogenic plant. Some of these are supported by historical record but some others are novels. Finally, is remarkable the necessity to make more ethnobotanical research on those cacti species that could be promissory resources in sustainable regional options to human groups that used them.

P2506. Exploring anthropological, historical and anatomical features of different tree-barks used for the manufacture of indigenous paper in Mexico

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This research focuses on the tropical tree-bark species used for the manufacture of Mexican indigenous paper. It discusses information about its historical and anthropological background and research results regarding the anatomy and histochemistry of the barks. This paper has deep cultural roots in Mesoamerica and recently has become a commercial handicraft product manufactured by the Otomi indigenous people in the Sierra Norte of Puebla region. Different aspects of the production process have been modified in order to respond to the growing demand. The bark is gathered from an increasingly expanding area within this region. New tree species have been adopted as source of bark which show anatomic and histochemical differences from the traditional ones used since pre-Hispanic periods. The latter implied changes of the traditional manufacture process with consequences in the quality and durability of the paper. The increasing intensity of bark extraction could affect the dynamic of the species populations, including *Trema micrantha* which shows a fast growing rate and is the most recent and widely used species for the manufacture of this indigenous paper.

P2507. Rural populations knowledge about *Borassus flabellifer* (L.) uses and their strategies to conserve it durably in southwest of Burkina Faso (West Africa).

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The vegetation landscapes of Burkina Faso southwest are dominated by *Borassus flabellifer*, a species which is greatly exploited by rural populations. This poster concerns people knowledge about its uses and populations strategies to conserve it durably. So, investigations have been made in this area.

The results revealed that each part of *Borassus flabellifer* is implicated in uses classified in five groups: food, craft, pharmacopoeia, building and others. About food, ripe fruits, mesocarp, buds, immature fruits, albumen, cotyledon and ashes from male inflorescences are consumed. In craft, leaves are used to make seven domestic uses objects. In buildings, trunks are used to make rafters, frames, parcels and animals' park enclosures. About pharmacopoeia, drugs made of young leaves, inflorescences, resin and mesocarp treat man sexual powerlessness, tooth and ear aches, dermatosis, parasites diseases. Some other uses are noticed: seats, bridges and hives are made of trunks.

This poster shows also rural populations strategies to conserve durably *Borassus flabellifer* by their methods to regulate plant densities and bot extraction.

P2508. Economic Botany Recapitulates Phylogeny in Yams (*Dioscorea* L.)

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Yams are a widespread and diverse genus (at least 450 species) of climbing monocots with underground storage organs. Diversity is highest in the seasonally dry tropics, in Madagascar, the Greater Antilles, Mexico, Northern Thailand and Southern Brasil. The underground organ (a rhizome or a tuber) of at least 70 species is used as food or medicine.

A phylogeny is presented which uses three plastid genes and 109 taxa from throughout the range of the genus for the first time. The tree topology indicates that different clades of yams have different types of use. Early branching clades, which mainly have shallowly buried rhizomes or perennial tubers, are used primarily as medicines. Their underground parts usually contain steroidal saponins, probably as a chemical protection against herbivory. It is these taxa that have been used to produce steroid drugs, including the contraceptive pill. The terminal clades, however, have deeply buried, annually replaced tubers. These taxa are used worldwide as a starch source; most tubers lack chemical protection. Thus it appears that in the genus *Dioscorea* plant morphological and chemical evolution has shaped human use.

P2509. Ethnobotany: Local Vegetables in Northeast, Thailand

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A survey of local vegetables in Northeast Thailand was conducted during 1999-2004. 197 species were found belonging to 67 families. Most of them grew in natural forest and swamps. One species was alga. Three species were ferns. Others were angiosperms. The biggest family was Leguminosae (25 spp.). The second one was Zingiberaceae (17 spp.). The third ones were Umbelliferae, Gramineae, Solanaceae, and Euphorbiaceae (9 spp. each). The plant parts mostly used were young leaves, shoots, fruits and flowers. Some can be fresh eaten. These vegetables can be used in two purposes - dipped and main dish. *Marsilea crenata*, *Centella asiatica*, *Anethum graveolens*, *Spondias bipinnata*, *Colocasia gigantea*, *Ottelia alismoides*, *Polygonum odoratum*, *Lobelia begoniifolia* and *Limnophila aromatica* were fresh eaten to go with chili paste. *Cleome gynandra* and *Crateva magna* were fermented before eat. Leaf crush of *Cyclea barbata* added with chili and fish sauce was delectable food. *Amorphophallus brevispathus* petioles, *Bambusa bambos*, *Wolffia globosa*, *Glinus oppositifolius*, *Caryota bacsonensis* and *Calamus diepenhorstii* young shoot were usually cooked for main dish.

P2510. Ethnobotanical Studies On The Tribes Of Puruliya District In The State Of West Bengal Of India

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The district of Puruliya in the state of West Bengal of India with its vast ethnic and vegetational diversity offers an immense scope for ethnobotanical study. This area is a heartland of many tribes for ages. The aim is to make a comprehensive account of ethnobotany of this principal tribal area which still nurses old practices but light of changes are seen not at the very distant far. So the need of the hour is to protect this tropical - ethnobotanical - archetypal treasure before it is lost forever.

The district is under the latitude 23° 42' - 22° 43' N, longitude 86° 54' - 85° 49' E, acquires an area of 6,259 sq. km. 'Tropic of Cancer' is passed through the north of this district. Topography is roughly undulating with terraced slopes. The place has a general monotony of 'Tropical Dry Deciduous Types' of monsoon vegetation. The vegetational belt is paleotropical. The object herein is to know the plants, plant parts, plant products have been used by the tribe for food, ropes and strings, dwellings, arms and weapons, furnitures, medicines, festivals and occasions, smokes and beverages, livestock and poultry farming, manual craft and culture, cultivation, and domesticity.

P2511. Ethnobotany of *Ficus* (Fig) species in Nepal

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Himalayan Journal of Sciences, Kathmandu, Nepal.

Ficus (fig) species are the most interesting group of trees, not only for their useful value but also for their growth habits and religious significance. They are considered as one of the principal sources of sustenance among the rural people. Present paper was focused to reveal the indigenous uses of *Ficus* (Fig) species in Nepal. Primary and secondary data were reviewed and assessed. Total three field visits were carried out in July 2001 and May 2003 in Dolpa district and March 2003 in Bardia district. Of total 36 *Ficus* species in Nepal, 16 were indigenously used for edible, fodder, fuelwood, vegetable, to medicinal use, etc. Ethnomedicine of *Ficus* (fig) species in Nepal was quite noteworthy. Eight fig species were used to treat 36 ailments. The contribution from the species *F. benghalensis* was the most possessing ingredients to treat 22 ailments. Some species such as *Ficus religiosa*, *F. benghalensis*, *F. benjamina*, etc. have a high religious value for both Hindus and Buddhists and are deemed sacred.

P2511a. Nourishing Plants Wild Utilized by the Ethnic Group Mixteco in Tepunte, Guerrero state, México.

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Tepunte is one site in the mountains of Sierra Madre del Sur in México. The community has a population of 330, most of them monolingual mixteco. Until an altitude of 850 m, with a climatic type weather half-moist. The mean annual temperature is 27°C and the pluvial precipitation is 1577 mm. The local vegetation consists of Oak forests with *Quercus glaucescens*, *Q. elliptica* and *Q. peduncularis* as dominant species. The aim of this project is to recover Tepunte's knowledge and natural resources management still used about nourishing plants wild. The methodological approach used is based on Gispert, 1979 proposal, with the following results: 24 nourishing plants species wild, these species are transformed in sauces, beverages, containers and wrappings for other food. The consumption of raw ingredients is widely found, and the fruits are the morphological fragments more used. These species are used daily at meals and on annual festivities, either religious or ritual. These results show that this ethnic group's traditional knowledge ancient about its environment it's still being acquired, transmitted and kept among adults, young and children of this Mixteca location.

P2512. Developing a useful antioxidant and antibacterial product from a *Combretum* species

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Antibiotic feed additives [AFA] are used in production of poultry and pigs. Resistance development may lead to human health problems resulting in EU banning of AFA from 2006. Screening of

trees indicated substantial antibacterial and antioxidant activity in leaves of a *Combretum* species. By using different extractants and bioassay-guided fractionation the most important antibacterial and antioxidant compound was isolated and characterized. It had an MIC of 16 µg/ml against *Staphylococcus aureus* and 125 µg/ml against *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The Trolox equivalent antioxidant concentration [TEAC] was 7.9 times that of Vitamin E.

The compound is present in high concentration in leaves and a leaf extract was potentiated by selective extraction to yield an extract with a TEAC value of 2.8 and an average MIC value of 0.08 mg/ml.

This extract was tested as replacement of AFAs in a poultry experiment. There was no significant difference in growth rate compared to the positive [bacitracin] and negative control, but there was an improvement in food conversion rates. If these results are confirmed it may lead to a commercially significant product.

P2513. Antioxidant capability of polysaccharides fractionated from mycelia of *Agaricus blazei* in submerged culture

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Five polysaccharide fractions (AB-1, AB-2, AB-3, AB-4, and AB-5) were obtained after a systemic solvent extractions and precipitations of *Agaricus blazei* mycelia with yields 5.20%, 9.03%, 2.84%, 17.47%, and 0.44%, respectively. Among which, AB-1 and AB-3 demonstrated great DPPH· radical scavenging ability around 85.0 % and 72.0 %, respectively, at a concentration of 5 mg/mL. Their ferrous ion chelating power were even more excellent at a concentration of 1 mg/mL, reaching almost over 99.65 % for fractions AB-2, AB-3 and AB-4 comparing to the reference control of citric acid (35%), at a dosage of 5 mg/mL, fraction AB-1 even showed 105% in efficiency. Moreover, GPC analysis showed that the more potent fractions were those with lower molecular weights: AB-1 (757 kD), AB-2 (887 kD) and AB-4 (631 kD). We found that the biological activities of various fractions are neither closely associated with the mean molecular weight nor pertinently related with the solubility. Apparently, the active functionality inherently borne in each individual structure and their actual availability must be acting the major role in these respects.

P2514. Classifying landraces of *Ensete ventricosum* in Ethiopia: traditional and experimental approaches confirmed

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Enset (*Ensete ventricosum*: Musaceae) serves as the staple or co-staple crop for 15-20 million people in southern and south-western Ethiopia. It is grown in home gardens under varying climates from 1300-3300 metres altitude among several ethnic groups. Leaf sheaths and corm provide starchy food which can be stored for a long time. Many landraces are grown. Farmers differentiate their phenotypes by size, shape and colour, and their site requirements. They distinguish three groups for consumption, and further uses for fibre, animal feed, or traditional medicine. Several landraces tolerate drought, frost, pests and diseases. With rising altitude, the number of cultivated landraces declines. Growing several landraces guarantees permanent food supply, and other products. Experiments on vegetative development, leaf anatomy and chemical content confirm the traditionally distinguished groups. In view of these results, systematics of the genus are discussed.

P2515. Usage of wild and garden plants in rural societies in surrounding of Magura National Park and Knyszynska Forest Landscape Park (Poland)

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The project aims at studying the uses of plants (wild and garden species) by rural residents. The studies focused on species affecting natural and cultural environment of Polish villages. The study areas are Magurski National Park and Knyszynska Forest Landscape Park, where industrial and urban development to date has not been intensive helping the traditional uses of plants to survive. Also important is the influence of Eastern ethnic groups: Lemko people in the Magurski NP and Belarussian people in the Knyszynska Forest LP, which retained their separate cultural and religious identity.

The studies are to provide information on uses of plants, e.g. as medicines, food, commercial crops and sacred items (blessed for later use), as well as variable household uses (repelling insects, weaving baskets or making brooms, applying for decorative purposes).

The rural communities undergo major cultural and social changes with traditional values and ways of life being replaced. The knowledge of plant species and their uses might help to preserve a vanishing part of rural culture. The project is currently in progress and its completion is planned for 2006.

P2516. Plants as estimates of civilization: first we take *Oedipus*, then we take Theophrastus...

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In ancient literature, plants are presented *via* a detailed description of their properties. It is quite possible that such information may connect early observations to current aspects of Plant Sciences. In *Oedipus at Colonus*, Sophocles (5th century B.C.) wrote about aesthetic values of plants surrounding a sacred spot "*this place is consecrate; it blooms with laurel, olive, vine, narcissus and crocus, rayed with gold*". While, Theophrastus (4th century B.C.), a student of Aristotle, argued that the features of every plant-tissue contribute to the prevailing traits of the species. His chapters are the most important botanical works that have survived from classical antiquity (i.e. in the books *Enquiry into Plants* and *Causes of Plants*). Examples: i) "*In some plants the flowers are more fragrant than the leaves, in others on the contrary it is rather the leaves and twigs which are fragrant, as in those used for garlands, e.g. myrtles like those used for garlands*". ii) The kinds of flavours, as of odours and colours are held to be 7: "*sweet, oily, bitter, dry-wine, pungent, acid, astringent, and the salty is also added to these as an eight*".

P2517. The value of non-coding genomic regions in the authentication of medicinal plant materials

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Molecular authentication provides an independent and effective means for identifying medicinal plant materials. Internal transcribed spacer (ITS) of rDNA and 5S rRNA spacer are commonly used regions for authentication. Our experience with *Dendrobium*, *Gentiana*, *Cinnamomum*, *Stemona* and *Strychnos* indicates that ITS is a stable marker that provides low intraspecific variation and moderate interspecific variation. On the other hand, 5S rRNA often show variation within an individual and may be used to differentiate between closely related species.

Some relatively conserved regions, such as TrnL intron and TrnL-F intergenic spacer in chloroplast genome, may be used to differentiate distinct genera. By comparing TrnL-F spacer sequences, *Solanum lyratum* can be distinguished from *Aristolochia mollissima*, an adulterant containing aristolochic acids. Amplification of chloroplast regions is less affected by fungal DNA contamination, adding advantage to using them for authentication purposes.

This work was supported in part by the Hong Kong Jockey Club Charities Trust (Project no. JCICM-2-02R)

P2518. Indigenous Healing Practices in Tibetan communities of Nepal: Review and Perspectives

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Traditional healers of Nepal practice folk herbal medicine in the rural areas of lowland and midhills, followed by Tibetan medicine in the high hills; whereas in the urban areas, Ayurvedic as well as modern medicine are commonly practiced. Ethnobotanical research conducted in the Tibetan communities of Nepal reveals that more than 400 species of native medicinal plants, including over 100 species of plants from lowland tropical zone, are used by Amchis to treat different ailments. Amchis or Tibetan doctors play a vital role in the Tibetan and Sherpa communities, where local people have no access of doctors and allopathic medicine. Interestingly, several species of medicinal plants used in Tibetan medicine are well-known ingredients of Ayurvedic medicine, and few species are used in folk herbal medicine too. Besides its significant role in primary health care, medicinal plants are income

generation source of majority of poor people. Over exploitation of some high value species, due to high demand in trade, are seriously threatened and needs special attention for their sustainable conservation and utilization for future generations.

P2519. Herbal Product Development in Thailand

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Similarity to other countries in Asia, Thailand, with her various geographic locations, is rich in the biodiversity especially medicinal plants. Traditional Thai medicine and Thai medicinal plants are unique with vast potential. The principle of herbal remedy is complex and involves many disciplines of sciences. Due to the world trend to use natural products for better quality of life, the National Research Council of Thailand started the new strategies for product-based research in the year 2004. An integrated project focuses on selected 5 multi-herb traditional formulas and 16 plants for monopreparation. The research emphasized on quality control of raw materials and their standardized extracts, *in vitro* and *in vivo* tests to ensure efficacy and toxicity. Clinical trails of potential well-formulated herbal products are in process. The establishment of production techniques of high quality plant material was also performed. All researched technology will be transferred to herbal industry. Those efforts are conducted for the benefit of mankind and the sustainable use of Thai herbal heritages.

P2520. Acaricidal activity of Thai medicinal plants

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Ethanol extracts from various parts of 19 medicinal plants collected in Thailand were analysed for acaricidal activity against *Dermatophagoides pteronyssinus*, the most common dust mite in Thailand. Extracts from 4 of the 19 plants showed significant acaricidal activity, the most potent being *Acronychia pedunculata* (stem bark) which killed 67% of mite after 72 hours exposure to 2.40 mg/cm² extract. Other effective plants are *Azadirachta indica* (seed, 60%), *Erythrophleum succirubrum* (stem, 55%) and *Trigonostemon reidioides* (root, 63%). The latter was tested at the applied amount of 1.44 mg/cm². However, *A. pedunculata* showed the high toxicity in the brine shrimp microwell toxicity assay, i.e., with the lethal concentration (LD₅₀) of 0.2 µg/ml. *E. succirubrum* showed the least toxicity (LD₅₀ = 245 µg/ml). Thus *E. succirubrum* is promising to be used as natural product acaricide for controlling house dust mites due to their high acaricidal activity and low toxicity.

P2521. Acaricidal activity of Thai medicinal plants

N. Soonthornchareonnon¹, V. Mahakittikun², W. Chuakul¹;

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P2522. Some important Medicinal plants of western ghats of India

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Western Ghats(WG) are situated towards West side of the peninsular India .WG is one among the three mega biodiversity centres of India.This hotspot region has a characteristic true Indian flora especially no. of endemic medicinal plants used for different ailments. Some of these plants are mentioned in Reg Veda

(4500B.C and 1600 B.C) and local healers of this area are still using few plants to treat no. of diseases . Around 1,500 endemic plant taxa are recorded from this area. Specially the rare, endemic and endangered medicinal plants like CARVIA CALLOSA(Acanthaceae),MAPPIA FOETIDA(Icacinaeae),ARDISIA RHOMBOIDEA(Myrsinaceae),DILLENIA BRACTEATA(Dilleniaceae) ,PTEROSPERMUM RETICULATUM (Sterculiacear) ,ACACIA WIGHTII (Mimosaceae), EUGENIA INDICA (Myrtaceae) , MEMECYLON SISPAENSIS (Melastomaceae) ,WRIGHTIA INDICA (Apocynaceae),PREMNA WIGHTIANA (Verbenaceae),Ixora lawsonii (Rubiaceae),BUCHANANIA BARBERI (Anacardiaceae), AGLAIA ELEAGNOIDEA (Meliaceae)and GLYCOSMIS MAURTIANA (Rutacear) etc. will be discussed

P2523. Asiatic medicinal plants acclimatized in Poland

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The importance and common use of *Platycodon grandiflorum* A. DC. and *Salvia miltiorrhiza* Bunge in Asia were the cause of the beginning works on their acclimatization in Poland.

It was found that *Platycodon grandiflorum* (*Campanulaceae* family) in Polish climatic conditions resists winter well and gives good crops of raw material (roots) and seeds. The germination of the seeds obtained in Poland was from 90 to100 % and they kept this ability up to 7 years. The raw material (*Platycodi radix*) contained from 3, 73 to 4, 53 % of saponins. Mean mass of fresh roots obtained from one plant after two years of cultivation was 55 g, after three years 145 g.

Salvia miltiorrhiza (*Lamiaceae* family) blooms and fruits in Poland. Its germination capacity is up to 60 %. The mean mass of fresh raw material (*Salviae miltiorrhizae radix*) from one plant after two years of cultivation was 11 g. Roots of *Salvia miltiorrhiza* obtained from cultivation in Poland contained similar amount of polyphenols and diterpenes as Asiatic commercial raw material.

This work - a scientific project - is financed by Polish Committee for Scientific Research.

P2524. Chemopreventive effect of embelia ribes in 20-methylcholanthrene induced animal model

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Chemopreventive effect of embelia ribes in 20-methylcholanthrene induced animal model Abstract

The methanol extract of *embelia ribes* fruit was evaluated for its chemopreventive properties. Chemopreventive effect of the extract was evaluated against 20-Methylcholanthrene induced fibrosarcoma. Oral administration of the extract at the doses of 200 mg/kg b.w. significantly increased the lifespan of the fibrosarcoma bearing mice and reduced the tumor volume when compared to that of untreated tumor bearing mice. Biochemical parameters such as thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and antioxidant enzymes such as glutathione-S-transferase (GST), glutathione peroxidase (GPx) and catalase (CAT) were estimated in the hepatic tissue. The extract at the dose of 200 mg/kg b.w. significantly restored the altered biochemical parameters. Thus from the present investigation it can be concluded that the methanol extract of *embelia ribes* exhibited significant chemopreventive properties

Key words: Chemoprevention, *Embelia ribes*, Antioxidants.

P2525. Impact of Invasive Weed *Ageratum conyzoides* L. on the Vegetation Structure and Dynamics in Himachal Pradesh, India

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Himachal Pradesh situated in northwestern India has a rich floral diversity owing to the variation in altitude (244-6750 m) and climate (from sub-tropical to temperate to alpine). However, during the last two decades a number of exotic invasive weeds have invaded thereby adversely affecting the native vegetation. Upon invasion these bring about irreversible changes in the structure and dynamics of natural communities. These weeds possess invasive characteristics such as fast growth, efficient resource utilization,

high reproductive potential, wide ecological amplitude and lack natural predators unlike the native species. In this context, *Ageratum conyzoides* (Asteraceae) is an invasive weed from tropical America that has invaded India and encroached upon agricultural lands, grasslands, pastures, and forests. It forms monospecific stands in the area of invasion at the expense of native plants and directly affects socio-economic condition and welfare of humans. The present paper attempts to describe the impact of invasion of *A. conyzoides* on the structure and dynamics of vegetation in the different ecosystems such as forest, grasslands and plantations.

P2526. Molecular Genetic Study of Invasive Species of *Heracleum* in Europe

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Tall species of *Heracleum* (giant hogweeds) were first introduced into Europe in the nineteenth century and are now widespread in many countries. There are three invasive species of the genus in Europe: *H. mantegazzianum*, *H. sosnowskyi* and *H. persicum* (or *H. laciniatum*). To elucidate genetic relationships between these species and to help locate the sources of origin of the invading plants we analysed samples from more than 100 populations collected in 19 European countries. We compared amplified fragment length polymorphism (AFLP) markers of three invasive species with two native species (*H. sphondylium*, *H. sibiricum*) and six Caucasian species. Our results confirm that *H. mantegazzianum* was introduced to Europe from the Caucasus and point to possible invasion scenarios. Our results did not suggest that *H. sosnowskyi* from Armenia and Europe were genetically close, indicating that this species in Europe either comes from other parts of the Caucasus, or that it was subject to some modifications (e.g. breeding) prior to its introduction. We suggest that *H. persicum* and *H. laciniatum* are synonyms in Scandinavia. Several cases of misidentification will be discussed.

P2527. USDA proposes revisions to plants for planting and propagation import regulations

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United States Department of Agriculture (USDA) proposes revisions to regulations 7 CFR 319.37 (known as Quarantine 37) governing imports of plants for planting and propagation to ensure adequate protection for agriculture, horticulture, forestry, and the environment against plant pests and noxious weeds. The modernization effort is prompted by plant imports transitioning from limited origin, fully treated, low numbered germplasm 'starter' material to plants of worldwide origin, rapidly distributed, and treated only when quarantine pests are found. Proposed regulations might include updated plant pest mitigation strategies; significant modifications to USDA regulatory approaches; and harmonized, transparent, consistent, and clear scientific and regulatory processes. Challenges include promulgating changes while simultaneously facilitating continued access to imported plant resources meeting strengthened US regulations for safety coupled with US commitments to international commerce.

P2528. Ecological Impact of the Invasive Shrub *Lantana camara*

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The bushes of lantana are highly floriferous and reproductively successful under favorable environmental conditions. The plant uses both sexual and vegetative reproductive strategies for propagation and proliferation. Lantana burns even when it is green and hence is a fire hazard. Cutting or burning of the aerial shoots leads to production of strong suckers and more vigorous growth. The plant has a heavily armored woody stem, opposite-deccusately arranged leaves and axillary, corymbose inflorescences. The flowers are of different colours depending on the variety as well as age of the flower. The role of butterflies and thrips in pollination and birds in seed dispersal further complicate the ecological impact of this invasive species. Lantana is a preferred host for the parasitic climber *Cuscuta*. This pernicious weed is responsible for

carrying thrips, which are phyto-phagous insects and *Cuscuta* to newer areas which it invades, consequently destroying the natural vegetation.

P2529. Ergasiophytes in the flora of Ukraine

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The aim of our study was to assess the influence of ergasiophytes on the natural vegetation. It has been revealed during the investigations that 210 species of plants can be classified as ergasiophytes by the mode of their immigration. By the naturalization status, there are 27 epecophytes and 53 agryophytes among them. Among agriophytes, there are 10% annuals, 30% herbaceous polycarpic plants, 10% shrubs, and 50% trees. Agriophyte trees (*Acer negundo* L., *Elaeagnus angustifolia* L., *Ailantus altissima* (Mill.) Swingle and others) are most dangerous for the natural plant communities; they are aggressively invading the meadows, steppe-meadows and abandoned pastures in the park. The invasives grow faster than the natives, shading their neighbors and thus stunting their grow. In some sites, these shrubs form extensive dense growths, resulting in the exclusion of other species. The shrubs affect natural areas by displacing native species, changing the vegetation structure and impeding its regeneration.

P2530. Invasive plants in Armenia

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Armenia is one of the newly independent states presently situated in the main transport route between Iran, Georgia and Russia. The very low activity of the State Quarantine Service has facilitated the entry of a number of new species of plants into Armenia which may become invasive weeds in natural ecosystems, and further invade the territories of other countries. Today we know about penetration and spreading here of some new invasive species of plants. Unlikely, at present, modern data on the spread and distribution of invasive plant species in the territory of the Republic of Armenia, and their impact on agricultural and natural areas are missing.

On the other hand, Armenia is the motherland for numerous species of plants, which have known as invasive weeds in different countries. There are 4 Armenian plant species in the list of "100 of the World's Worst Invasive Alien Species" (ISSG/IUCN). More than 30 species are growing in Armenia, and at the same time they are very dangerous weeds in the North America. Investigation of potentially invasive species in Armenia may be very important for biological control in different countries around the World.

P2531. Invasive plants in Ukraine

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Highly invasive species are defined in this study as aliens characterized by stress-tolerant strategies, active dispersal in large areas, high competitive ability, persistence of populations, and potential ability to invade semi-natural and natural plant communities.

Highly invasive species are represented in the Ukrainian flora by at least 91 species (0.9% of the alien fraction), including 24 archaeophytes and 66 kenophytes. By naturalization, they are subdivided into agriophytes (19 species), epecophytes (66), and ergasiophytes (6). Annuals, xeromesophytes, and species of North American origin prevail. According to invasive ability, 5 groups are distinguished: (1) species able to occupy the whole country - 20 spp.; (2) aggressive species in different phytogeographical regions - 31; (3) aggressive species with local distribution - 12; (4) species occurring in the whole Ukraine in all suitable ecotopes, but mainly in man-made habitats - 18; and (5) potential aggressive species - 10.

P2532. The role of tree-fall gaps in the invasion of exotic plants in forests: the case of *Rubus phoenicolasius* Maxim (Rosaceae) in Maryland, USA

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Invasive plants often enter forests via tree-fall gaps, but can invade an entire stand only if individuals either establish or persist under closed canopy. We evaluated the role of gaps for *Rubus phoenicolasius* in forest stands in Maryland, USA. A native of Asia, *R. phoenicolasius* is invasive in disturbed sites and successional forests in eastern USA.

We censused 15 x 15 m plots centered in gaps created in a 2002 storm vs. random points in adjacent young and old deciduous stands. In the old stand, established *R. phoenicolasius* ramets were present in 10 of 20 gaps, but only 2 of 19 random plots. Ramet density and cane length were greater in large v small gaps, and tip-rooting was limited to large gaps. In the young stand ramets were in all 4 gaps and 4 of 5 random plots.

Seedlings and fruits were frequent in the young stand. In the old stand seedlings were only in 10 gaps and fruiting occurred only in 3 large gaps.

Thus, tree-fall gaps are required for seedling establishment and fruiting in mature forest. We are investigating whether established ramets persist with canopy closure in the forest and under different shade treatments.

P2533. The Impacts Caused by the Invasion of *Prosopis juliflora* (SW) D.C. over the Structure and the Diversity of Trees in the Caatinga Vegetation, Northeast Brazil

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Prosopis juliflora (SW) D.C. was introduced in the Brazilian semi-arid in the 40s. It was broadly divulged but it invades the caatinga, mainly gallery forests, sites which retain a great biodiversity and endemic species. In order to study this problem, invaded and non invaded sites were selected in four counties: Monteiro and Taperoá (I and II) in the state of Paraíba; Carnaúba dos Dantas and Acari (III and IV) in Rio Grande do Norte. In each county twenty 400 m² plots were used and 5,151 adult individuals were identified. Non invaded sites showed 35, 32, 37 and 15 trees species and Shannon-Weaver index values (H'): 2.81; 2.74; 2.33; 1.96 for I, II, III and IV. The invaded sites showed: 15, 11, 04 and 06 species and H' values of 0.56; 0.80; 0.24; 0.14 for I, II, III and IV, respectively. Values of importance (VI) and cover (VC) for *P. juliflora* were: I) 212.2 and 183.7; II) 184.50 and 162.80; III) 245.2 and 189.66 IV) 245.59 and 183.09. A few native species resist to *P. juliflora*: *Capparis flexuosa* L. and *Cereus jamacaru* DC. Typical native species do not occur in the invaded areas: *Anadenanthera macrocarpa* (Vell.) Brenan; *Astronium urundeuva* (Fr. All.) and *Schinopsis brasiliensis* Engl.

P2534. Contrasted population dynamics of a dangerous, endangered species in alien and native populations

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Range changes and extinctions are natural processes at evolutionary time scale. However, human activities may alter them profoundly. For instance, an endangered species in one place may become an invasive alien in distant regions.

Rhododendron ponticum L. (Ericaceae) is a Tertiary, relict species with a disjunct, narrow distribution restricted to both western and eastern ends of the Mediterranean basin. In the last interglacial, western populations occurred as far North as Ireland, but this species went extinct from the British Isles in the last glacial maximum. In 1763, it was re-introduced for gardening purposes, and it currently behaves as an aggressive alien in British natural communities, threatening biodiversity.

A Markov Chain approach was used to analyse population dynamics of this species in both alien and native locations. Remarkable demographic differences were found not only between both kinds of populations, but also between coastal and inland alien stands.

This predictive modelling approach will help to assessing extinction risk of native populations and potential invasiveness of alien stands in the face of Global Climate Change.

P2535. Combating Noxious Exotic Weed *Mikania micrantha* in the Field

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Mikania micrantha is a noxious alien weeds with a slightly woody vine species belonging to Asteraceae. It was introduced to Hong Kong as early as 1884 and now spread far beyond Hong Kong. *M. micrantha* was able to use shrubs and small trees as its support and then smothered the supports with thick mantels of its shoots. Physical removal experiment showed that uprooting all the creeping stems completely was difficult. The remained creeping stems sprouted in a short time and some of the slashed stems revived under considerable precipitation and the effect could only maintain for several months. Sulfometuron methyl was an effective herbicide to kill the weed. *Cuscuta campestris*, a parasitic plant, was able to suppress of *M. micrantha*. No other species was found to be dead caused by *C. campestris* within the infected circle. Restructuring plant community with multi species planting was conduct. Regional, fast growing, broad canopy and tall tree were criteria for selecting plant species. Within two years, the canopy of the community became continuous and suppressed the underneath weed, especially *M. micrantha*, indicating the effectiveness of the restoration measurement.

P2536. Root growth and environment, The carbon cost of root function in boreal forests

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The first function of the root is to take up the surplus of carbohydrates produced by the shoot. Even in fine roots there is a high level of stored carbohydrates (Persson 2000): The gravity turns the direction growth by the movement of water vapour inside and outside of plants. The upward growth is connected with water supply for the elongation growth from inside, the downward from outside. The root body is protected by the root cap against too early water uptake. The root cap becomes often asymmetric and in consequence the water uptake and water loss in the elongation zone is asymmetric too.

Data from various European forest stands suggest annual turnover rates of 2-4 times the average fine-root biomass. The root function is extremely costly and is enhanced by a high carbohydrate supply. The aboveground capacity to supply carbohydrates by photosynthesis governs the long-term root and mycorrhizal growth rate. Roots can grow in any direction (Kutschera et al. 2004). Carbohydrates are stored at high levels in the fine roots; starch levels of 25-30 % are frequently found. Tree roots can persist in the soil and survive for several years even when photosynthesis is restricted.

P2537. Roots and pseudobulbs of lithophytic orchids

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Root and pseudobulb anatomy of twenty orchid genera has been understood (mountains of Elephants Chain, Cambodia). Many of study species are lithophytic plants (60%). Generally, they have stem origin pseudobulbs, composed of 1-3(-5) internodes (Bulbophyllum, Chelonistele, Coelogyne, Eria, Flikingeria, Liparis, Pholidota genera). One of few species, who has pubescent pseudobulb is *Eriodes barbata*. The pseudobulb has three separated meristematic poles. One of them gives the leaves, the second - the inflorescens, and the third - the new bud. Majority of lithophytic orchids, being protected by the moss, have the green succulent roots, pasted often by pair on the rock. The long pause in life cycle of this orchids decides for development in roots and stem not only reserving, but the photosynthetic and the mechanical tissues too. The chlorenchyme and the aerenchyme are formed in their median parenchyma. In exterior parenchyma and epiderme are present numerous raphids or druses. Uncommon in this region terrestrial orchids (5%) have also the greenish pseudobulbs, who are this adaptation for the survive during the forest-fire (*Arundina* and *Spathoglottis* genera).

P2538. The root growth in the young forest ecosystems of Central Siberia

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To estimate annual production of fine tree roots in the forest ecosystems of main forest-forming species in Siberia we used ingrowth method.

The results of our research showed that the rate of root growth for coniferous tree species is in 7 times faster than for deciduous species.

The root growth was the fastest at the first year of the experiment. First year growth is the potential possible rate of root growth for these species under natural conditions. And all differences of root growth rate depend on species features because it was under equal environmental conditions for all cases.

In the following years the rate of root growth decreased. Mean annual tree roots growth rate made up 86.9 - 42.4 - 13.7 - 97.1 - 19.4 - 9.1g/m² for arolla pine, Scots pine, larch, spruce, aspen and birch, accordingly. Annual root death made up 3.1-4.6% of annual growth for coniferous species and 2.3-14.4% of annual growth for deciduous species.

The fine roots stock in the young forest ecosystems is annually renewed on 21.1-24.2% under coniferous stands and on 2.9-4.4% under deciduous stands.

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P2540. Effects of copper stress on root system morphology and topology of *Cannabis sativa* plants grown in hydroponic culture.

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Root system morphology is genetically determined and varies among species and individuals; it can be modified by many environmental factors, including nutrients, water availability, biotic and abiotic stress (as heavy metals). Copper (Cu) is a micronutrient, toxic for plants at high concentrations. Hemp (*Cannabis sativa*) has a potential role in phytoremediation of heavy metals but till now its ability to tolerate and/or accumulate copper has been scarcely studied. The aim of our work was to investigate the influence of two copper concentrations on the root system architecture of hemp (*C. sativa*) plants grown in hydroponic culture, using morphological and topological analyses. The presence of copper at high concentrations (50µM and 100µM) in the nutrient solution induced a drastic and highly significant reduction of all the considered parameters (e.g. root, stem and leaf fresh weights, total root length, root system branching, total root surface and projected area, mean root diameter). Moreover copper treatments influenced the topological pattern of the root system, that in treated plants was clearly herringbone-like.

P2541. Available Micro Nutrients as related to soil physical and chemical properties.

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Profile distribution was investigated for DTPA-extractable Zn,Cu,Mn and Fe in relation to depth and important soil characteristics in ten representative soil profiles of Hissar. There was specific pattern of distribution of available Cu,Mn and Fe due to alluvial nature of soils. A decreasing trend for available Zn with increasing depth was observed. Mean values for available Zn,Cu,Mn and Fe were .27 ,.81,4.38 and 2.98 PPM respectively. Distribution of Mn was influenced inversely by soil pH,EC,ESP and CaCo3 content. Distribution of Fe and Cu also was influenced inversely by ESP. Distribution of Cu was influenced inversely by CaCo3 .Organic carbon content controlled dominantly of all the four Micro-nutrients.Cation exchange capacity (CEC)content controlled dominantly only the available Cu.Four multiple regression models to predict the available Micro-nutrients(Zn,Cu,Mn and Fe)OF a soil from organic carbon and CEC is also presented. The amount of correlation was 46,53,42 and 41 respectively.All the soil samples were deficient in available Zn,medium in Cu and low to medium in Mn and Fe.

P2542. Biological control of common root rot of wheat (*Bipolaris sorokiniana*) by *Trichoderma* isolates

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Trichoderma viride T112 and *T.viride*(MO), *T.harzianum*(M) and *T.harzianum*T 194 were used as potential biological agent for control of common root rot caused by *Bipolaris sorokiniana*. Cell free and antifungal metabolite produced by all *Trichoderma* isolates inhibited growth of *Bipolaris sorokiniana*. The inhibition varied among isolates of *Trichoderma* and ranged from 58.2 to 93.93% using the cellophane overlay method and from 66.66 to 98.25% in volatile test. Mycelial growth of *B. sorokiniana* was numerically reduced more by *T. viride* T112 than the other isolate tested(P<0.01). The ability of *Trichoderma* isolates to reduce the incidence of common root rot of wheat in the glasshouse condition was investigated. The seed soaking treatment and also soil treatment with *T. viride* T112 and *T.viride* (MO) were the most effective in reducing infection by the pathogen compared with the healthy control (P<0.01). All isolates of *Trichoderma* plus pathogen or alone increased plant height, fresh and dry weight of root and shoots of wheat seedling compared with the corresponding controls. Among *Trichoderma* isolates, *T.viride*T112 and *T.viride*(MO)) proved to be the best supporting growth of wheat (P<0.01).

P2543. Portuguese Ethnobotanical Artefacts Collection at Beja Botanical Museum

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Botanical Museum of the Polytechnic Institute of Beja has a collection of artefacts made with Portuguese plant raw materials that have been assembled in the last three years with the support of the Ford Foundation (Portugal). The collection also includes the plant raw materials.

The artefacts were obtained in all regions of Portugal, including Madeira and Azores Islands.

The collection includes musical instruments (*Fraxinus angustifolia*), baskets, hats and finger protections (*Arundo donax*), cork spoons (*Quercus suber*), spoons (*Erica arborea*), baskets (*Secale cereale*, *Rubus ulmifolius* and *Salix viminalis*), dolls (*Zea mays*), sculptures (*Ficus carica*), decorative items (*Allium cepa* and *Allium sativum*), embroideries (*Gossypium* sp.), fans and brooms (*Chamaerops humilis*), toothpicks (*Salix* sp.) and others.

These artefacts will be used as reference vouchers for future studies on Portuguese Ethnobotany.

P2544. Tolerance of vascular plants in anthropogenic habitats of Ulan-Ude city

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Anthropotolerance - a private example of phytotolerance or stability of plants. In author opinion any estimation of anthropotolerance should be based on mathematical parameters, and not just on visual estimation. Proceeding from this as such parameter we choose activity of species (A) which connects in itself "occurrence or ecologo-coenotic amplitude".

We make attempt to connect a parameter of species activity in various anthropopressure zones of Ulan-Ude city with the certain values of a gemerobie scale.

As a result of our researches it is revealed, that values of plant activity can vary widely on anthropopressure zones and in case of Ulan-Ude city do not reach the greatest possible size 22,4 points. It is observed, that in spite of the fact of increase floristic variety in wooden building subzones, connected with the presence of unhumiliated sites of plant cover, nevertheless there are same high active species, as in stone building subzones.

P2545. Anti-inflammatory effects of total extract of curcuma amada in a rheumatoid arthritis rat model.

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Previous studies have suggested efficacy of parenteral administration of *Curcuma amada* in animal models. In this study, topical effect of total extract of *Curcuma amada* (CA) on chronic inflammatory response induced by Freund's Complete Adjuvant in rat was studied.

The adjuvant contained heat killed mycobacterium tuberculosis suspended in mineral oil (Arlacel A) and light paraffin. Injections were made in the right ankle foot and tibio-tarsal joint region of rats. On day 15 following adjuvant administration, animals were treated with 100 mg/kg dose of the total alcoholic (50%) extract on paw daily for 20 days. Body weight, arthritic index, joint diameter and blood level of TNF-alpha were recorded during the experiment and at the last day of treatment. The data indicated the alcoholic extract could significantly ($p < 0.05$) reduce joint diameter (29%), arthritic index and blood level of TNF-alpha (61%) of treated animals compared with control group. The data points out to the potential efficacy of topical administration of CA extract in rats.

P2546. Developing of apple scab warning services in Uttaranchal Himalayas

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Effect of temperature, rainfall, leaf wetness and relative humidity known to exert a powerful influence on scab (*Venturia inaequalis*) development, was studied under field conditions during 1993 to 2004. 608,4 to 672,6 degree-days were necessary for discharge of 50% of the ascospores. PAD were varied from 0 to >720000 from place to place in Uttaranchal Himalayas. In some of the place the PAD was very low (<1000), there were only two sprays were required at the end of the primary inoculum. In epidemic year 1996 at Bhatwari fruit belt, reducing one fungicide applications in the year under reference. In the warning system the DMI curative fungicides were used in orchards at many places of Uttaranchal hills. The same fungicides were also applied at short intervals in the protective treatment. The warning treatment under these conditions gave a reduction of spraying frequency upto 3 to 4 times compared with regular protective spray. It is concluded that the use of warning equipment (μ METOS) together with a curative fungicide gives a sufficiently safe and effective control of apple scab in Uttaranchal Himalayas. Detailed results would be presented.

P2547. The Effect Of Three Different Planting Areas And Corm Size On Some Morphological And Biological Characteristics And Yield Of Saffron (*Crocus sativus* L.) In Kocaeli Province Of Turkey

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Saffron (*Crocus sativus* L.) is a cormous plant has the most economic value as medicinal and aromatic ingredients world wide. The study was carried out with 3 different planting areas (field, greenhouse and plastic tunnel) and 2 different corm size A (10-27 mm) B (28-45 mm) in 2003-2004 growing season, under Kocaeli (Turkey) climatic conditions.

According to the statistical data; the longest harvest period was obtained from greenhouse-B corm size with 56 days; the highest flowering was 2.66 flowers/plant in greenhouse-B corms; the tallest leaf length was 47.17 cm/plant in greenhouse-B corms; the highest leaf number was 29.25 leaves/plant in greenhouse-B corms; the highest rate of saffron fresh yield was 4691 g/da obtained from greenhouse-B corm size; the highest rate of saffron dried yield was obtained from greenhouse-B corms with 877.53 g/da; the highest daughter corm rate was 4.26 corms/plant obtained from greenhouse-B corms; the highest daughter corm size was obtained from plastic tunnel-B corms as 29.619 mm/corm. Dry saffron yield was harvested from greenhouse (465.765 g/da) that was higher than the yield obtained both from the field and the plastic tunnel.

P2548. Development of descriptor list for evaluation genetic resource of service tree (*Sorbus domestica* L.)

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Service tree (*Sorbus domestica* L.) belongs to introduced species in Slovakia. Most of trees succeed age more than 200 years. Adapted population is possible to use in food industry, pharmacy,

landscaping and in wood-working industry too. For all that evaluation of population for purpose to determination variability quantitative and qualitative characters on trees, leaves, flowers, fruits and seeds level was realized. At leaves studied length range 120 - 331 mm, width range 42 - 163 mm and length of leaf stalk range 19 - 95 mm. Fruits length range 14.3 - 38.2 mm, width range 13.1 - 41.2 mm and weight range 2.9 - 36.1 g was found. Observed seeds had length in range 5.2 - 9.1 mm, width range 4.0 - 6.3 mm and weight range 0.02 - 0.24 g. It was found strong variability in shape of leaves, fruits and seeds. Experimental data and image documentation is used on development of descriptor list for evaluation species *Sorbus domestica* L. It makes possible characterise genotypes and determined genotype differences.

P2549. Micropropagation of the medicinal plant *Stemona tuberosa* Lour.

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Stemona tuberosa Lour. is a medicinal plant belonging to the Stemonaceae family. It has been used for the treatment of respiratory diseases in China and other East Asian countries for a thousand years. The indiscriminate collection of wild growing populations is increasing every year, which is particularly alarming because the important alkaloids are located in the rhizome of the intact plant. Vegetative propagation techniques have been developed but many problems arise due to infections and the time consuming process. In vitro propagation might allow the rapid production of disease free plantlets for field culture.

A micropropagation protocol of *S. tuberosa* was developed using shoot culture. Shoots were induced from *in vitro* shoot and node explants on solid Murashige and Skoog (MS) medium. For the multiplication step shoots were cultured on MS medium supplemented with various types and concentrations of cytokinins. Several combinations of BAP and IBA were tested, too. Shoot multiplication was best when 20 μ M BAP were used. Regenerated shoots were successfully rooted on free hormone MS medium and plantlets could be established in the greenhouse.

P2550. Asymmetric fusion between protoplasts of *Helianthus annuus* L. and UV-treated protoplasts of *H. mollis* L.

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Being one of the four most important oil crops in the world, sunflower is the subject of numerous breeding programs. Enlarging its genetic base will allow it to be better adapted to environmental conditions. Wild sunflower species served as genetic base for the modern sunflower, carrying traits as disease resistance, cytoplasmic male sterility.

H. annuus line HA300B protoplasts were isolated from 7-days old hypocotyls and *H. mollis* protoplasts from mesophyll of *in vitro* grown plants. The isolation and purification of protoplasts was according to Krasnyanski and Menczel (1995) protocol. *H. mollis* protoplasts were irradiated with UV light (2 μ mol m⁻² s⁻¹) for 5 to 60 minutes and the efficiency of the treatment/protoplast viability were determined.

The protoplasts from the two species were mixed at a density of 4x10⁶ protoplasts/ml and electrofusion was induced using 3 pulses of 50 μ sec and intensity 500V/cm. The fusion products were immobilised in agarose droplets and cultivated according to Krasnyanski and Menczel (1995).

Up to this stage of experiments several hybrid calli were regenerated, some of them showing signs of regeneration (green buds on the calli).

P2551. Influence of Mannitol and Sucrose on the Olive In-vitro Culture for Iranian Cultivars (Zard and Dezful)

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Carbohydrate source and its concentration play a significant role in improving olive proliferation procedure. This paper compares the impacts of two carbohydrates, mannitol and sucrose on microshoots growth of Iranian olive cultivars Zard and Dezful. For this purpose, initially, we collected uninodal explants removed from

3-year greenhouse grown plants. The cultivars explants were individually cultured in OM media containing sucrose or mannitol (18 and 30 g/l), augmented with 2iP (4 mg/l). Then, we performed the first explants subculture in OM media with 2iP (4mg/l), containing mannitol or sucrose (30 g/l), after five weeks. The second subculture was accomplished employing OM media containing mannitol or sucrose (30 g/l), supplemented with 2iP and BAP (1 and 2 mg/l) separately or compoundly. The results of this research work are as follows. For Dezful cultivar mannitol (30 g/l) supplemented with 2iP (2 mg/l) provides the best proliferation results in the all steps. For Zard cultivar in the initial step sucrose (30 g/l) and in the rest of successive subcultures mannitol (30 g/l) are suitable carbohydrate sources.

P2552. *in vitro* germination of seeds of *Puya raimondii* harms (Bromeliaceae)

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Seeds of *Puya raimondii* were collected, on July 2002, from Calipuy located in Santiago de Chuco, district of La Libertad - Perú. Sow was held on July 2004, so seeds had 2 years of dormancy, although is known that this kind of seeds only have one year of viability. The basic medium was composed for inorganic salts used in hydroponics, plus thiamine HCl 100 ppm, riboflavin 40 ppm, pyridoxine HCl 40 ppm, nicotinamide 400 ppm, sucrose 3% (w/v), agar 0.8% (w/v) and combination of next hormones IAA (0, 0.5, 1, 1.5 ppm) and GA₃ (0, 0.5, 1, 1.5 ppm) in a factorial design, pH and electric conductivity were adjusted to 5.6 and 2.3 mS, respectively, before sterilized by autoclaving at 121°C and 15 lb/plg² for 15 minutes. Seeds were disinfected with sodium hypochlorite 1.5% and rinse 3 times with sterile water and sowing was held in a chamber sterilized with UV-C radiation, after that, tubes were carried to chamber for germination at 16°C ± 2°C in darkness. Germination occurs 10 days after sow, becoming to 55 % of germination in media supplied with GA₃ 1 ppm and IAA 0.5 ppm. Moreover, we demonstrated that this seeds can germinate after 2 years of dormancy.

P2553. Analysis effects of different factor on regeneration and transformation of three Iranian cultivar of tomato (*Lycopersicon esculentum*)

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Abstract

Tomato (*Lycopersicon esculentum*) is one of the most important vegetable crops and a genetic model for improving other dicotyledonous crop plants. However, tomato transformation is still not routine nor reliable. In this research effect of three genotypes (KalG, Kal early, Su2270 as the commercial cultivars in Iran), two explants (cotyledon and hypocotyls), growth regulators (BAP 2mg l⁻¹, zeatin 2mg l⁻¹) and two strain of *Agro bacterium tumefaciense* (pGV3850 and LB4404) on regeneration and transformation of tomato plants were investigated. In this transformation method, no feeder layers of tobacco, petunia or tomato suspension cultures were used. Our results showed there were significant differences between genotypes and explants in shoot number formation. KalG had maximum shoot number in cotyledon and Su2270 had maximum shoot number on hypocotyle. Maximum shoot regeneration was obtained with zeatin 2mg l⁻¹. Transformation rate was 6% for cultivar Kal early to 10% for cultivar KalG. Also it seems strain pGV3850 of agrobacterium was more efficient compared LB4404. Transformation events were confirmed by Gus assay and PCR analysis.

P2554. Micropropagation of *Hippeastrum puniceum* (Lam.) Kuntze (Amaryllidaceae).

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The Amaryllidaceae family includes numerous Brazilian species with ornamental potential, particularly *Hippeastrum puniceum* (Lam.) Kuntze, an herbaceous plant with attractive flowers. This genus occurs from Mexico all the way to Argentina, and Brazil is home to 20-30 *Hippeastrum* species. *H. puniceum* has not been domesticated, so little is known about its propagation. Micropropagation is a valuable technique for production of high quality plants, to intensify multiplication and to speed up propagation of target species. The aim of this study was to develop

a micropropagation protocol for *H. puniceum*. Different explant types, growth media and growth conditions were evaluated. Bulb sections and leaf discs were excised and cultivated on Murashige & Skoog (1962) growth medium supplemented with different growth regulators, at various concentrations. The cultures were maintained under a 16 hrs light photoperiod and 25 ± 2°C. Bulb sections were highly responsive and produced 4-5 bulblets/section introduced *in vitro*. The bulblets developed into normal plants after transplanting to pots containing a proper substrate and transfer to the greenhouse.

P2555. Establishment of an *in vitro* amyloplast inducing system using cultured tobacco BY-2 cells.

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Amyloplasts are starch-containing plastids that play important roles for both plant carbon metabolism and human diet. To examine the molecular mechanisms controlling the amyloplast development, an amyloplast inducing system in cultured BY-2 tobacco cells was used. In the present study, we examined the relationship between starch-storing cell development and phytohormones on amyloplast formation. As a result, auxin addition repressed amyloplast formation, whereas, cytokinin addition accelerated starch accumulation, regardless of the timing of hormone addition. In addition, reduction of endogenous cytokinin, lead to a reduction of starch accumulation. We also found that amyloplast can be reconverted into proplastids by auxin addition. All these changes are correlated with the changes of starch synthesis gene expressions. These studies clearly demonstrated that auxin and cytokinin exert opposite effects on amyloplast formation by regulating the starch synthesis gene expression, and we propose that this simple amyloplast induction system provides an ideal model for analyzing the process of differentiation and dedifferentiation of amyloplasts.

P2556. Effect of abiotic stress on regeneration and psoralen content in a medicinal plant, *Psoralea corylifolia*

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World wide soil salinity has become a major agricultural threat. Substantial areas of earth's potentially productive lands are affected by salinity and alkalinity. Considering the importance of salt tolerant plants in reclamation of the wastelands it is beneficial to adopt biotechnological approaches to raise stress tolerant plants of varying economic utilities. Experiments were conducted to develop salt tolerant plantlets of *Psoralea corylifolia* (Fabaceae), a potential source of psoralen and isopsoralen used in the treatments of leucoderma, leprosy psoriasis and inflammatory diseases. The *in vitro* cultures were subjected to various concentration of NaCl (0-400 mM). NaCl incorporated in MS medium supplemented with IAA + Kn + Ad + CH promoted growth, percent regeneration, number of shoots and leaves till 75 mM NaCl. Psoralen quantified through HPLC exhibited 2-fold enhancement at 75 mM NaCl. Higher concentration of NaCl inhibited shoot and root growth and gradual reduction in psoralen. Proline accumulated in salt tolerant cultures as a sequel to salt stress.

P2557. Zygotic embryo culture of *Pinus heldreichii* and *Pinus peuce*

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Pinus heldreichii (Christ.) and *Pinus peuce* (Gris.) are important, endemo-relict, high-mountain pines of Balkan Peninsula. These two pines are adapted to dry and very hot summer with intensive solar radiation, also to the cold conditions of the winter period. Such ecological characteristic enabled them to build powerful belt of forest vegetation, but today very degraded by human activities. *Pinus heldreichii* and *Pinus peuce* are usually propagated by seed, but seed production in natural stands is limited. Zygotic embryo culture has to be method to study the factors that control growth and differentiation of embryos. The aim of this study was to elaborate factors influencing the conversion of isolated mature zygotic embryos of *Pinus heldreichii* and *Pinus peuce* into

plantlets, and to determine the influence of different nutritional factors to this conversion. Culture of isolated zygotic embryos was established to improve protocols for successful somatic embryogenesis of these two pines.

P2558. *In vitro* propagation and conservation of Thai Zingiberaceous medicinal plants

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The project was performed to apply plant biotechnology for the production and conservation of Zingiberaceous medicinal plants. We established efficient protocols for *in vitro* propagation of *Curcuma longa*, *Gagnepainia thoreliana*, *Kaempferia parviflora*, and *Zingiber petiolatum*. The explants were cultured on MS medium supplemented with high concentrations of plant growth regulators (PGR) prior to transfer to PGR-free MS medium. Under these conditions, the shoot induction rates of 6-34 shoots/explant were obtained after 12-20 weeks of cultures. We also developed a protocol for conservation of 5 species, i.e. *Curcuma longa*, *Kaempferia galanga*, *Hedychium ellipticum*, *Zingiber petiolatum*, and *Zingiber niveum*, using slow-growth conditions. *In vitro* cultures could be effectively maintained for 6 months at 25°C on MS medium supplemented with mannitol at concentrations of 1-3% as a growth retardant. Shoots were subsequently recovered and multiplied on MS medium. Rooting was spontaneous achieved. Rooted shoots were successfully transferred to soil and grown in the field conditions.

P2559. Endemic *Aster albanicus* species: a case study on its tissue culture and possible genetic changes induced

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Aster albanicus D., an endemic endangered plant was analyzed to find the optimal conditions of propagation and possible genetic somaclonal variation imposed by "in vitro" culturing via RAPD markers.

The explants were organized (meristems, apical and lateral buds) and unorganized systems (leaf pieces). The media were: proliferation of organized explants [MS/2, ANA:GA₃ (1:1)], subculturing [BAP:ANA (10:1)], rooting [AIB:GA₃ (3:1)]. The leaf pieces were inoculated in MS, BAP:ANA (1:10). The opposite ratio was applied for the meristemoids and the regeneration of plantlets. Direct organogenesis resulted in high proliferation. Young buds of different origins showed differences in their indicators. Medium for meristems was optimal for buds; plantlets derived from apical buds developed better. A quick formation of adventitious shoots was observed. AIB induced a high index of rooting. 94.7% of pieces had calli after five weeks, and meristemoids appeared at 92% of the calli in six weeks. The high ratio BAP/ANA stimulated a big number of adventitious shoots. The fingerprinting analysis showed no genetic polymorphisms among naturally and "in vitro" made plants.

P2560. Studies on growth and productivity of *Asparagus racemosus* under in vivo and in vitro conditions.

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Studies on growth and productivity of *Asparagus racemosus* (Willd.), Shatavari, an important medicinal and ornamental plant have been conducted under in vivo and in vitro conditions. Steroidal saponins, shatavarin I-IV are the active ingredients of the plant is present in tuberous roots. Different plant growth regulators and N, P, K caused a significantly (P<0.001) increase in the number and size of tuberous roots of *A. racemosus*. Growth was found maximum in the plants treated with IBA (50 ppm) + P2O5 (160 mg.kg⁻¹), where as it was reduced significantly (P<0.001) in the NAA (50 ppm) treated plants. NAA proved best at lower concentration, 10 ppm. In vitro multiple shoot formation was observed maximum in the MS medium supplemented with kinetin (2.0 mg.l⁻¹) + NAA (0.1 mg.l⁻¹). Plants thus raised have been transferred to field conditions which showed over 80% survival. Quantitative estimation of protein contents was also dramatically

influenced. Activity of invertase, acid phosphatase and peroxidase was found to be change in in vitro grown plants.

P2561. Somatic embryogenesis in heliconia from sections of ovary and zygotic embryos

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This work aimed to establish protocols for the induction of somatic embryogenesis in heliconia species. A first experiment used sections of ovaries of *H. chartacea* Lane ex Barreiros cv. Sexy Pink in MS medium with combinations of indole-3-acetic acid (AIA: 0; 1; 1,5; 2 and 2,5 mg/L) and 2,4 - dichlorophenoxyacetic acid (2,4-D: 0; 3; 4 and 5 mg/L). On the second experiment were used zygotic embryos came from immature and mature fruits of *H. bihai* cv. (L.) L. Lobster Claw Two in MS medium with combinations of AIA (0; 1 and 2 mg/L) and 2,4-D (0; 5; 10; 15 and 20 mg/L). Sections of ovaries, treated with 2,5 mg/L AIA and 5,0 mg/L 2,4-D showed the presence of embriogenic cells distinguished by their small size, isodiametric shape and densely cytoplasmatic appearance in observations at stereomicroscope, besides citochemical analysis. Scanning electron micrograph (SEM) showed somatic embryos at globular stages from the embriogenic callus. At the second experiment the formation of somatic embryos took place just in zygotic embryos came from mature fruit cultivated at the absence of growth regulators.

P2562. Production of hGM-CSF by Transformed Root Cultures of *Digitalis purpurea* L.

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Due to their fast growth rates and biochemical stability, transformed roots (or hairy roots) remain unsurpassed as the choice for model root system and have promise as a bioprocessing system. Granulocyte-macrophage colony-stimulating factor(hGM-CSF) is a glycoprotein that stimulates the production of granulocytes, macrophages, and white blood cells. In this study, we describe the possibility that transformed root would be a good host for the production of clinically useful protein. Transformed root lines were established after the inoculation of transgenic foxglove plantlets expressing hGM-CSF with *Agrobacterium rhizogenes* ATCC 15834. hGM-CSF levels in the biomass and medium were quantified by ELISA. For transformed root grown in MS medium, the maximum level of hGM-CSF after 6 weeks in shake-flasks was 0.83 mg/L or 0.42% total soluble protein; up to 11.3% of hGM-CSF was secreted into the medium. Total hGM-CSF levels were increased by addition of polyvinylpyrrolidone and gelatin to the medium. hGM-CSF production by transformed roots had a negligible effects on growth compared with transformed roots of wild-type plants.

P2563. Somaclonal variability of plants regenerated from long-term embryogenic tissues of wheat (*Triticum aestivum*)

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Wide spectrum variability of plants features (R₀, R₁, R₂ and R₃ progenies) regenerated from long-term calli of wheat five genotypes and three hybrid combinations (Kazakhstan selection) has been revealed, including the useful changes on the terms of maturation, stems height, parameters of productivity and leaf width. It was established, that the character of features changes depends from initial genotype: the early ripening lines in R₂ somaclones of c. Celynnaya - 3S, short stem forms and forms with high graining heads in somaclones R₂ and R₃ of c. Otan, antocian colour of leaf ears in somaclones R₁ c. Kazakhstanskaya - 25. In spite of morphological changes the most of somaclones were identical with profiles of storage proteins of initial genotypes. At the same time was found: zero mutation on the 1D chromosome in glutenins profiles at R₁ somaclone of hybrid line H-4 and differences on -, - zones in gliadin profiles at somaclone R₁ of c. Kazakhstanskaya - 25. The terms of phenotypic display of somaclonal mutations in regenerated plants can be decreased by the increasing of initial callus tissues subculture number.

P2564. Anti-microbial action of the essential oil of *Lippia gracilis* Schauer

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Antimicrobial activity of the essential oil (EO) of *Lippia gracilis* Schauer over fungi of vegetable tissue culture and heliconia endophytic bacteria were evaluated. For the fungi *Geotrichum candidum*; *Trichoderma viride*; *Torula herbarum*; *Paecilomyces* sp.; *Fusicoccum* sp.; *P. aeruginens*; *Curvularia lunata* *Aspergillus nidulans*; *A. flavus*; and *A. Niger* mycelium discs were inoculated in PDA medium with oil (0; 420; 440; 460; 480 e 500 mL/L). The inhibition percentage in a 420 mL/L was 95,58% and 89,40% for *C. lunata* and *A. niger*, respectively. For the other fungi, the percentage was 100%. A suspension of the *Salmonella choleraesuis-diarizonae*, *Enterobacter asburiae*, *Bacillus thuringiensis*, *B. pumilus*, *B. cereus*, *Klebsiella pneumoniae*, *E. hormaechei*, species was added to the NYDA medium solid, in Petri plates. In each plate wells were drilled and filled with water or EO (420 µL/L). Was assessed the diameter of the inhibition halo. *K. pneumoniae* was bacterial species less sensitive and *E. hormaechei* was more sensitive.

P2565. Influence of methyl jasmonate on 4'-Demethyl-6-methoxypodophyllotoxin and 6-methoxypodophyllotoxin accumulation in a *Linum tauricum* ssp. *tauricum* (Willd.) cell culture

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The lignan podophyllotoxin (PTOX) is used for the semi-synthesis of the anti-cancer drugs etoposide, teniposide and Etopophos®. PTOX is restricted to only a few plant species. Because of its expensive chemical synthesis and limited supply, other sources for this rare natural lignan are required.

Here we report the initiation of conventional callus and suspension cultures of the Balkan endemic species *Linum tauricum* ssp. *tauricum* Willd., and the production of the PTOX derivatives 4-demethyl-6-methoxypodophyllotoxin (4-demethyl-MPTOX) and 6-methoxypodophyllotoxin (MPTOX) as the main lignans. MPTOX was readily identified by comparison of its HPLC retention time (11.95 min) and UV spectrum with that of an authentic sample. The second isolated lignan (8.68 min) differed from MPTOX in the absence of the 4'-O-methyl group. The levels were 0,516 mg/g dw and 0,127 mg/g dw respectively.

Experiments with addition of extracellular methyl jasmonate showed an increase of the biosynthesis of the two compounds, which is indication that lignans may be plant defence compounds and could be a useful tool for the enhancement of lignan production.

P2566. The induction of androgenic embryos of *Aesculus flava* Marshall.

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Induction of androgenic embryos of yellow buckeye (*Aesculus flava*) was achieved on solid MS medium (Murashige and Skoog, 1962), supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin (1.0 mgL⁻¹, each). Medium for embryo multiplication consisted reduced concentration of 2,4-D (0.01 mgL⁻¹) and the same concentration of kinetin (1.0 mgL⁻¹). The development of androgenic embryos was asynchronous, so that embryos at their globular, heart-shaped, torpedo-like and cotyledon stages were observed in the same culture. Androgenic embryos *A. flava* with normal morphology were cultured on solid hormone free medium for embryo germination. Secondary embryos appeared on the radicle of androgenic embryos grown on the hormone-free medium. The goal of the work was mass production of haploid androgenic embryos from anther culture and induction of secondary somatic embryogenesis and plantlets formation of *Aesculus flava* in order to develop efficient *in vitro* regeneration methods to be used in genetic transformation experiments applicable in the pharmaceutical industry.

P2567. *In vitro* studies on the effect of red rot toxin on the callus of sugarcane (var. CoC 671)

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Red rot (*Colletotrichum falcatum* Went) of sugarcane has become a threatening disease for the early maturing variety CoC 671. It causes death of cane stalks and also results in low juice and reduced sucrose. There are studies on the effect of *C. falcatum* on organic metabolites of susceptible cane, while there are no reports on the effect of red rot toxin on sugarcane callus. During the present investigation, metabolic changes induced by red rot toxin was studied using callus of susceptible sugarcane cultivar CoC 671.

The pure pathogen of *C. falcatum* grown on oatmeal agar was used for toxin extraction. Sugarcane var. CoC 671 callus tissue was induced using MS medium. Different concentrations of partially purified toxin were incorporated into the MS medium (0.05- 0.5%). The flasks were inoculated with 2-3 g of friable callus of CoC 671 and were kept for incubation for 50 days and were analyzed for organic constituents such as protein, total and reducing sugars, polyphenols and proline. Result show that there was increase of protein and polyphenols content of toxin treated callus, while there was a reduction of total and reducing sugars and proline as compare to control.

P2568. Somatic embryogenesis induction and plant regeneration in carob (*Ceratonia siliqua* L.)

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Ceratonia siliqua (carob) is a leguminous tree that grows in some regions of Portugal (Algarve) and in other countries of the Mediterranean basin. Propagation of this species by cuttings is problematic because rooting is difficult to achieve. In this context, *in vitro* tissue culture techniques may offer alternative ways of dealing with the problem of carob propagation. In this work somatic embryogenesis induction and plant regeneration in carob is described. Somatic embryos were induced on immature cotyledons of several cultivars. Best results were obtained when 4.4 µM BA and 0.5 µM NAA were used in the induction medium. Media supplementation with 5 mg/l dithioerythritol increased somatic embryo (SE) formation. SE conversion was obtained after treatment with gibberellic acid and kinetin. Poor root development was observed in the converted embryos and, in most of the cases, a further treatment with IBA was necessary to induce root formation. Histological and ultrastructural studies showed that the structures formed were in fact SE showing an independent vascular system and the accumulation of reserve compounds identical to those found in zygotic embryos.

P2569. *In vitro* propagation and flowering in the ornamental bamboo *Bambusa wamin*

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Studies have been conducted on *in vitro* multiplication of *Bambusa wamin* using mature nodal explants obtained from nearly 15 years field grown clump. After surface sterilization with 0.15% mercuric Chloride solution the nodal explants were cultured on appropriate nutrient medium. Mature nodal explants of *Bambusa wamin* produced multiple buds *in vitro* when cultured on Murashige and Skoog (1962) medium containing 5.0mg/l 6-Benzylamino purine. Rapid shoot multiplication occurred on MS medium supplemented with 2.0mg/l BAP and 0.8mg/l Kn. *In vitro* flowering occurred in cultures that were maintained *in vitro* for nearly 18 months. In addition to normal green shoots occasional formation of albino shoots was also recorded. More than 95% of *in vitro* shoots rooted on modified MS medium supplemented with IBA (7.5-10.0 mg/l) in 7-8 weeks. The *in vitro* raised plantlets after hardening and acclimatization showed 85% success on transfer to field. The micropropagated plants in field exhibited similar morphology as the mother plant with bulbous internodes.

P2570. *In vitro* morphogenetic studies in *Azadirachta indica* A. Juss

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The *in vitro* culture studies are helpful for improving plant quality. The improvement of trees through tissue culture is more significant as conventional methods of breeding are not of much use due to height, long life span, heterozygosity, etc. However, somatic embryogenesis, micropropagation and production of haploids can be useful in selecting and developing superior species. For any

successful approach it is necessary to develop a successful regeneration system using suitable explant. In this species anther-derived callus is highly regenerative. For shoot regeneration MS medium supplemented with various concentrations of benzyladenine (BA) alone or supplemented with NAA is most suitable. Somatic embryos were produced during second sub-culturing of callus on MS + 1 mg/l+0.1mg/l NAA or MS + 0.5mg/l BA medium. The chromosome number in regenerated roots and callus showed various ploidy levels. For checking browning of shoots, roots and callus PVP was highly effective. Thus anther can be used to develop haploids and for genetic transformations.

P2571. A protocol for micropropagation of the *Lilium ledebourii* (Baker) Boiss an endangered rare species endemic to Iran

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The perennial plant *Lilium ledebourii* (Baker) Boiss (Liliaceae) is an endangered rare species endemic to Iran. It is under surveillance of regional Environmental Protection Agency. Due to the collection of these plants, specially of the underground organs, the number of this species is continuously decreasing. Effect of growth regulators, sucrose concentration and scale segment on bulblet regeneration were investigated. The highest number of bulblets was formed on a MS medium with BA (0.1 mg l⁻¹) + NAA (0.1 mg l⁻¹) and 9% sucrose at basal segment. The presence of growth regulators increased fresh weight of bulblets compared with hormone free medium. The bulblets at the end of the culture period were given cold treatment at 4 °C for 8 weeks and then transplanted to a potting mixture of sand, leaf mold and peat moss (1:1:1). The horticultural habits of the cloning plants are being investigated to introduce the germplasm into lily breeding program

P2572. *in vitro* Germination of *Gentiana olivieri* Griseb. Seeds

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Gentiana genus is grown widespread on the world. It is used as a traditional medicine in many countries because of bitter secoiridoid glycosides in their roots. *Gentiana olivieri* Griseb. grows in Middle Anatolia and Southeast Anatolia and used for the reducing blood glucose level in diabetic patients especially in Southeast Anatolia. It is obtained that this activity is caused by isoorientin. Common usage of *G. olivieri* results in extinction risk because of collection from natural habitats too much. In this study, it is aimed to reproduce *G. olivieri* by *in vitro* germination with tissue culture techniques.

MS (Murashige&Skoog) and MS with WPM (Woody Plant Medium)'s macronutrients were used as the base media. Different concentrations (0.0345, 0.069, 0.139 mg/l) of Gibberellic acid (GA₃) as a plant growth regulator media and 1.01 mg/l KNO₃ added media and hormon-free media were used for the *in vitro* germination of *G. olivieri* seeds.

Approximately 400 seeds were sown each of these media aseptically and then *in vitro* germination ratio were determined. The best result obtained from the media of MS medium with WPM macronutrients with 0.069 mg/l GA₃.

P2573. Micrografting of neem (*Azadirachta indica* A.Juss.) shoot apex onto persian lilac (*Melia azedarach* L.) rootstock seedlings

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Neem (*Azadirachta indica* A.Juss.), native to the arid regions of Indian subcontinent, is a valuable tree because its fast growing rate and many technological important applications. We examined micrografting of the highly interested *A.indica* onto *Melia Azedarach* L. (Persian lilac) rootstock seedling, with the objective of estimating the resistance of this union to cold treatment afterwards. In these micrografting experiments the age of rootstock, the localization of graft and hormonal treatments during the time course of growth were examined under *in vitro* condition and after transferring to soil. The best shoot tips graft success was found on young rootstock in lateral hypocotyls position. Exogenous

auxine in association with cytokinine was necessary to the best union and growth. If salicylic acid showed an inhibitory effect on the graft-take, salicylic acid treatment cause the highest grafting success, signifying a useful role of salicylic pretreatment in acclimatization to soil conditions for these grafted plantlets.

Key word: *Azadirach indica*, Auxine, Benzyl amino purine, cytokinine, *Melia azedarach*, Micrografting, Salicylic acid

Footnotes

P2574. Effect of calcium on growth and phenolic accumulation in cell cultures of *Hypericum androsaemum* L.

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Hypericum androsaemum L. (Hypericaceae) has been used in folk medicine owing to the diuretic and hepatoprotective activities of its leaves. The pharmacological properties are attributed to the several phenolic compounds found in the plant.

Suspension cultures were established from hypocotyl-derived callus using liquid MS medium supplemented with 3% sucrose, 1 mg l⁻¹ 2,4-D and 0.5 mg l⁻¹ BA. Cultures were kept at 25±2°C under a 16-h photoperiod.

The growth kinetics, over a 16-day period, was typically sigmoidal, with cells entering the stationary phase 12 to 13 days after inoculation. Cellular contents of flavonoids, hydroxycinnamic acids and total phenols declined during the first week of culture and thereafter increased gradually with time, reaching a maximum on day 14.

Cell extracts from 14-day-old cultures proved to be active superoxide radical scavengers, as assessed by evaluating its ability to inhibit superoxide-driven reduction of nitro blue tetrazolium.

Culture of cells for 7 or 14 days in nutrient media containing high concentrations of CaCl₂ caused a slight reduction in growth and a substantial increase in the accumulation of flavonoids.

P2575. Plant regeneration from protoplast in Banana (cv. Dwarf Cavandish)

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We investigated if the isolation & regeneration of protoplast from leaves tissues banana (cv. Dwarf cavandish) was isolated from mesophyll tissue by enzymatic digestion in 28 and 32 H with 25°C conditions and cultured using a semi-solid medium. Initiation protoplasts frequency was 90% in optimal conditions. Protoplasts were transferred to MS liquid medium without hormone. Under these conditions, protoplasts remained virtual and active. We observed cell wall formation and cell division after 5 days. Callus of frequency formation was 50-70% in MS liquid medium supplemented with BAP (0.005 mg/l) & IAA (0.002 mg/l) , calluses were 50-100 in diameter, after 4 weeks. Calluses were cultured on MS semi-solid medium supplemented with BAP (3 mg/l) & IAA (2 mg/l). Shoot regeneration efficiency was 30% via organogenesis among 143 isolated callus within 28-42 days. They showed further development of the dividing protoplasts. We succeeded isolating protoplasts from mesophyll in Dwarf cavandish. Regeneration was mentioned.

This report reveals young leaf material banana Cv. Dwarf cavandish is very recalcitrant towards regeneration from protoplasts of mesophyll tissue.

P2576. Antimicrobial Activity of Static Cultures of *Asparagus racemosus*.

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The static cultures of *Asparagus racemosus* from shoot and root explants were generated in Murashige and Skoog's (MS) medium supplemented with various growth hormones. Genetically transformed callus culture was also established by co-cultivation of leaf explants with *Agrobacterium tumefaciens* in MS medium. The in-vitro cultures developed were maintained for six month and tested for presence of various phytoconstituents. Antimicrobial activity of methanolic extracts of in-vitro cultures was carried out and compared with that of natural plant using disk diffusion method against gram positive and gram-negative bacteria. In the present

investigation it was observed that the static cultures generated from plant have great potential to produce secondary metabolites present in natural plant. Methanolic extracts of root and root callus (150 µg/ml) possess significant antimicrobial activity against gram-positive bacteria.

P2577. Callus production of 3-hydrogenkwadaphnin, a potent new anti-leukimic agent

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A novel compound of *Dendrostellera lessertii* (Thymelaeaceae), named 3-hydrogenkwadaphnin, shows potent anti-proliferative activity especially on the leukimic cells. In order to complete our knowledge about the mode of action of this new compound and also to initiate clinical trails and other pharmaceutical investigations, large quantities of this compound are needed. Based on this limiting factor, alternative ways of 3-hydrogenkwadaphnin production should be worked out. In that respect, we evaluated its callus production under different experimental conditions and from various explants. Excised tissues (leaves and stems) of *D. lessertii* were cultured on M.S medium supplemented with various concentrations of 2, 4-D and Kinetin, and the optimum callus growth and maintenance for leaf and stem explants were established. Although we have been successful in callus production of 3-hydrogenkwadaphnin, but more extensive research is required to maximize the production level.

P2578. Application of mutation breeding technique for producing NaCl tolerant plants of banana in tissue culture and greenhouse conditions

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To study of possibility to induce salt tolerant clones in banana by using mutation technique, an experiment was conducted with factorial (gamma irradiation and salt concentration factors) in a CRD design. In this research, plantlets of banana cv. Dwarf Cavendish were produced by subculture of irradiated shoot tips. It deserves to mention that consequent subcultureing was aimed at getting rid of chimera. Next, these explants were transferred to MS medium containing 2.5 mg.l⁻¹ BAP and NaCl concentrations of 0, 6, 7, 8, 9 g.l⁻¹ for 2 months. Then, living buds were transferred to medium without salt. After one month, we repeated the first stage. All living buds rooted and transferred to potted soil. Acclimatized plants were irrigated weekly with above NaCl solution. Other irrigation, was done with free-salt water. There was also a negative relation between salt concentration and survival - proliferation. In second salinity stress, salt had not significant difference on survival percentage. No-significant difference of effect salt on survival in second salinity stress.

P2579. Molecular instability of poplar somatic clones (*Populus nigra*) at SSR loci

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Somatic clones of black poplar (*Populus nigra*) were developed from callus cultures of petioles of aseptic shoot origin and grown up in field. Molecular instability of the somatic clones developed from two trees N-SL and N-309 was determined by five microsatellite markers. Twenty SSR alleles were detected, the number of alleles per marker ranged from 1 to 6, with an average of 3.3 detected by ALF (automatic laser fluorometer). A dendrogram (SPSS11) based on the presence versus absence of alleles discriminated the groups of somatic clones (1 to 35) of N-SL from somatic clones of N-309. The polymorphic markers of WPMS2 (5 alleles), WPMS4 (6 alleles) and WPMS20 (6 alleles) revealed somatic variation in 1 clone (37) out of the 6 from the N-309 tree, and three subgroups out of the 29 somatic clones from the N-SL tree (17 and 24), (2 and 14) and (10 and 15). The new SSR-clones may provide new genetic resources to improve molecular diversity of poplar. *Source of grants: OTKA-M-04563, OECD-JA00018642, USA-Hungarian Fulbright Commission, and SzÖ-4/2003.*

P2580. Effective factors on the shoot and microtuber formation of potato under *in vitro* conditions

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The efficiency of *in vitro* shoot and microtuber formation of two potato commercial cultivars from single nodal explant was studied on solid and liquid MS- basal medium applying different inoculation culture density.

The experimental design was complete randomized blocks with three replications.

The observations on number of shoot and microtuber per erlenmeyer flask were recorded 4 and 10 weeks after culture explants respectively.

The results revealed that the number of microtubers as well as shoots were affected by the genotype, culture media and also inoculation density of culture. The highest produced microtubers belonged to Agria cultivar. Comparative studies between solid and liquid culture media revealed that shoot and microtuber formation were more efficient in solid medium. Mean comparison also showed that there is significant differences among three levels of inoculation density, so that 6 and/or 9 explants per erlenmeyer were superior compared with density of 3 regarding both trails under study.

P2581. Plant regeneration *in vitro* of *Rhodiola rosea* L.

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Rhodiola rosea L. (Crassulaceae) is a valuable medicinal plant. It is a perennial grass of quite limited distribution. Over the last years *Rhodiola rosea* L. area decreases drastically because of intensive exploitation. Biologically active compounds of *Rh. rosea* are widely used in pharmaceutical industry. Therefore the species is included in the Red Book of Bulgaria and other countries.

The purpose of our investigation was to elaborate efficient method for *in vitro* propagation of *Rhodiola rosea* L.

Various nutrient media and explants were tested. Explants isolated from *in vitro* germinated seeds were more efficient for plant regeneration than explants isolated from plants grown up in nature. This system for *in vitro* micropropagation allows, both, reservation of this endangered species and providing biotechnology approach for production of valuable secondary metabolites.

P2582. Events of dedifferentiation during callus induction from *Rubia tinctorum* L. leaves

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Callus induction is usually the first act at biotechnological protocols. Formation itself is often described but events of dedifferentiation are usually not observed.

We have investigated the early steps of dedifferentiation process. Sterile leaves were placed on MS medium and the harvested samples were observed by electron microscope, HPLC and TLC to follow the changes. We have found that chlorophyll content of leaf pieces decreased gradually while amount of anthraquinones increased. Not only concentration of the anthraquinones has changed but the composition was altered too. The most sweeping changes in the ultrastructure appeared in mesophyllar and vascular bundle sheath cells. Chloroplast structure (size of grana, starch content, number and size of plastoglobuli), amount and vacuolization of cytoplasm were altered obviously, however, even two weeks after induction chlorenchymatic cells showed no signs of divisions. Presence of proplastids and dense cytoplasm were characteristic after a few days in the transfer cell like cells. First cell divisions appeared near to venation and typical yellow cells of calli were formed at these particular places.

P2583. Embryogenic callus induction in *Stemona tuberosa* Lour

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Stemona tuberosa Lour. has long been recommended in Chinese traditional medicine for the treatment of respiratory diseases. Utilization of this plant has been largely as crude drugs and the traditional propagation techniques are not economic because of low efficiency. Production by *in vitro* culture might be an interesting alternative. The results presented here suggest potential of

embryogenic callus induction with further application to produce large plantlets via somatic embryogenesis on a large scale. Investigations trying to obtain the best media for callus induction were performed by employing different types and concentrations of cytokinins. The amount of compact callus was positively correlated with the concentrations of BAP and TDZ. The best results were obtained with 20 μ M TDZ. Friable callus developed from compact callus on MS media supplemented with 5 μ M 2,4-D, 5 μ M BAP, 5 μ M TDZ and 5 μ M IBA. In the third step embryogenic callus was achieved in liquid MS medium supplemented with 3 μ M 2,4-D in the dark with the subsequent formation of somatic embryogenesis.

P2584. Investigation the effect of different planting bed and the position of stem nodes on the production of minituber from tissue culture plantlets in two varieties of potato

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The tubers from "Marfona" and "Desiree" after growing till 15-20 cm in height in greenhouse were topped and transferred to thermotherapy conditions. After four weeks, the meristems were excised and placed on Paper Bridge in liquid media and were tested for virus free. Then for propagation, three liquid media were used: MS (free hormones), MS+0.5 mg/l BAP, MS+0.5 mg/l NAA. The free hormone media produced vigorous plantlets with suitable rooting. Then for planting beds, the position of stem nodes and minituber production, apical meristem of stems and roots were removed and stems were divided to Bottom, Middle and Top and cultured on four beds: peat moss/perlite 3:1, turf/perlite 3:1, leaf mould/perlite 3:1, rice hull/turf 1:1. All beds except leaf mould/perlite 3:1 produced desirable plants. The position of nodes had effect on the ratio of compound leaf to total leaves. After harvesting minitubers and measuring the traits, rice hull/turf 1:1 planting bed, in all trait was better. In this stage, also the position of nodes except in the interaction of position of nodes x planting bed on total minituber weight had no significant effect on minituber production.

P2585. Effect of Cryopreservation Techniques on Regeneration and Genetic Stability of Potato Genotypes

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Most of the researchers in cryopreservation concentrated on vitrification technique. Results showed various potato cultivars (Agria and Picasso in this study) react differently to this method, so that; there is not a unique protocol for several cultivars. Results of preculture stage: Application of high sucrose content (0.7 M) enhances the post-freeze recovery percentage, in most cases, short duration of preculture is preferred, ABA in this stage (0.5-3 mg/l depended-cultivars) had positive effects on post-thaw recovery and regeneration. But application of ABA on donor plants had no significant effect, proline had significant effect. Results of dehydration: DMSO is not proper for lowering free water of potato meristems' tissues, concentration of cryoprotectant must be about 7M and proper duration of dehydration, without and with loading solution is 50 and 40 min., respectively. Increasing the rate of freezing and thawing steps by aluminium vials instead of polypropylene vials improved the post-thaw recovery. Also, some of freeze-induced damages in newly thawed tissues, related to free radicals which are produced from photochemical reactions.

P2586. Temporary dynamics of hyoscyamine contents in the different parts of *Datura innoxia* Mill. hairy-roots.

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Using HPLC the hyoscyamine content was determined in the young (1 and 2 cm from the root tip), intermediate (3-6 cm) and old (6-10 cm) parts in 1-, 4-, 15-, 19- and 21-day-old hairy-roots. The active growth and/or tissue differentiation were observed at the stages of the 4 and 15 days. At the 21 day the growth was finished, and alkaloid content was highest in all parts of the root, but it was decreased after the transfer to the new medium (1-day-old roots). In the intermediate and old parts hyoscyamine amount was restored fast and remained practically constant at all other stages. In the young parts it was almost two-time less than in the

old ones at the stages of active growth. However, after growth stops the alkaloid content was increased gradually and became similar in all parties of the root. The ultrastructural features of cortex cells related to alkaloid amount changes were observed. The biosynthesis localization, accumulation and transport of tropane alkaloids in hairy-roots were discussed.

P2587. In Vitro Prediction for Salt tolerance in wheat

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CALLUSES of three salt tolerant wheat genotypes and their hybrids were grown on MS medium supplemented with different NaCl ppm. Growth rates of calluses decreased as the salinity levels were increased in the culture medium. There were highly significant differences among the tested genotypes and their hybrids in response to salt stress in callus growth rate. The results showed that callus growth rate and plant regeneration of hybrids were less decreased as salt concentration was increased compared with that of parents. Sakha-69 and hybrid (Sak. x Giza) were the most tolerant and scored high percentage in plant regeneration. Proline content increased parallel to the increase of salinity. The results prove that the genetic differences in wheat salt tolerance could be tested early *in vitro* and the more the genotype is able to produce proline under the stress conditions, the more it will tolerate salinity.

Keywords: Wheat, Prediction, Tissue culture, Salt-tolerance, Regeneration, Proline.

P2588. The utilization of embryo culture in the interspecific hybridization between *Cucumis anguria* and *Cucumis zeyheri*

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Wild *Cucumis* species, *C. anguria* L. and *C. zeyheri* Sonder, could be used as donors of various characters in cucumber (*C. sativus*) improvement and breeding. Both wild *Cucumis* spp. are mutually cross-compatible, however there are some physiological and genetical barriers. These obstacles can be overcome by embryo-rescue culture. The embryos were excised from the fruits of *C. anguria* one to six weeks after pollination by *C. zeyheri* and were cultivated on five different culture media (MS-medium supplemented with ascorbic acid, caseinhydrolysate, coconut water and gibberelic acid). Vital plants were recovered from the embryos from fruits at least two weeks old. The hybrid characters of regenerated plants were proved by isozyme analysis (H 1.1., H 1.2., H 2). Morphological assessment of parental genotypes and plants issued from their crossings was performed continuously during the plant growth. The most significant traits indicating hybrid character of plant F₁ generation were till now (stage of beginning of flowering) localized on the shape of leaf blade. This research was supported by grants: 1) NAZV No. QF 4108 (MA CR); 2) MSM 153100010; 3) MSM 6198959215.

P2589. Effects of Brassinosteroid on Cotton Regeneration

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The effects of brassinolide (BR) on cotton somatic embryogenesis were examined. 10 day-old cotton (*Gossypium hirsutum* L. cvs., Cooker 312 and Nazilli M-503) seedlings were used as explant source and hypocotyls were cultured on MS basal medium (MS salts, vitamins, 30 g l⁻¹ sucrose, 2.2 g l⁻¹ phytigel, 1 g l⁻¹ polyvinylpyrrolidone, 1 mg/l 6-benzylaminopurine, 2 mg/l kinetin) for callus induction. After one month, proliferated calli pieces were collected and cultured on the same media containing various concentrations of BR (0.1, 0.5 and 1.0 μ M) in complete dark conditions at 26 °C. Differential somatic embryogenesis rates arising from BR treatment were detectable within 10 weeks. In 0.5 μ M BR containing media for Cooker 312, somatic embryogenesis was earlier compared with control. For Nazilli M-503 both direct root and somatic embryo formation were observed in MS basal medium containing 0.5 and 1.0 μ M BR, within 8 weeks. Embryos at different stages of development and plantlets were collected for molecular analyses of putative BR-responsive cDNA's. The data indicates BR plays a role for induction of somatic embryogenesis and root regeneration from calli.

P2590. Studies on Genetic stability of Micropropagated Iranian Date Palm Plantlets by RAPD MarkersP. Eshraghi^{1,2}, R. Zarghami³, H. Ofoghi¹;¹Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Islamic Republic of Iran,²Department of Biology, Khatam University, Tehran, Islamic Republic of Iran, ³Agricultural Biotechnology Researching Institute of Iran, Karaj, Islamic Republic of Iran.

Phoenix dactylifera L. (Aracaceae) is an important source of economic revenues. The slowness of conventional methods has made *in vitro* tissue culture an alternative method for propagation. Identification of possible somaclonal variants at an early stage of development is considered to be useful for quality control in plant tissue culture. RAPD based detection of genetic polymorphism is useful in describing somaclonal variation in plant species. The aim was to compare genetic stability between offshoot and derived plantlets via somatic embryogenesis of Iranian cultivar "Khanizi". Characterization of regenerated palms was performed using 10 selected primers. Of these 10 primers, 4 (40%) primers enabled the identification of polymorphism. DNA polymorphism was investigated on different palms through the analysis of mother palm and regenerated from several clones. Data were recorded as presence or absence of amplified products, correspondence analysis was performed using NTSYSpc-2.02e. The estimation of genetic similarity coefficient based on RAPD band-sharing date indicated that six regenerated plants were more than 90% similar to the mother plant.

P2591. Plant Regeneration from Immature seeds Embryons-Derived Embryogenic Calluses of *Vinca herbaceae*A. Ataei Azimi¹, B. Delnavaz Hashemloian²;¹Azad Islamic University, Saveh, Islamic Republic of Iran, ²azad islamic university, Saveh, Islamic Republic of Iran.

Vinca herbaceae is herbs with two either opposite or whorled entire leaves, Iranian indigenous and it is growing native in north of Iran. Immature seeds are used for culture in this investigation. Immature seeds embryos formed small calluses at a frequency of 30% on MS medium supplemented with 1.5mg/l (2,4-D) with 0.1mg/l (kin) after 8 weeks of culture. After a second subculture using MS basal medium at 6-week intervals, small calluses formed a small quantity of yellowish, compact embryogenic calluses. Upon transfer to MS basal medium supplemented with 0.5 mg/l 2,4-D and 0.5 mg/l kin, embryogenic calluses gave rise to numerous somatic embryos. Embryogenic calluses cultures developed into plantlets without roots growth at a frequency of 100% when plated onto MS basal medium. The growth rates and morphological characteristics of plantlets were different. 1-The most plants have normal shape and normal growth. 2- Succulent shoots plantlets have unnormal shapes and little growth speed. 3- Plantlets with 3 leaves in any nod have normal shape and normal growth.

P2592. Effect of precursors feeding on essential oil production and molecular identification of clove, *Eugenia caryophyllus* callus cultures.M. A. Matter¹, R. S. Farag², F. A. Ahmed², M. K. El-Bahr¹, S. A. Ghaneem¹, M. R. Rady¹;¹National Research Centre, Giza, Egypt, ²Faculty of Agriculture, Giza, Egypt.

Callus cultures were established *in vitro* from leaves explants of aseptically grown *Eugenia caryophyllus* seedlings. The obtained calli were grown on induction medium supplemented with different concentrations of phenol or p-cymol or salicylic acid. The results clearly showed that high concentration of phenol or p-cymol in the medium resulted in the highest essential oil percentage. Qualitatively, phenol or p-cymol at concentration 160 mM is the most suitable level to produce an essential oil comparable in its composition with the already known clove essential oil. SDS-PAGE analysis for callus tissues grown in induction medium with different levels of phenol or p-cymol showed over-expression of the protein band of approximately 30 KDa. However, RAPD analysis revealed high percentage of polymorphisms within clove callus tissues grown on medium containing phenol, p-cymol, salicylic acid. **The variation in banding patterns were observed between control treatment and other treatments.**

P2593. A procedure for *in vitro* plant regeneration from seed-derived callus

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A procedure for *in vitro* plant regeneration from seed-derived callus was developed for *Arctotis arctotoides*, a plant noted for its several medicinal uses among the rural people of the Eastern Cape province in South Africa. Callus induction was initiated in basal MS salt supplemented with 3% sucrose (w/v) and 1% agar (w/v) at different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D). After two weeks, morphogenic responses were evident in cultures and deeply stained light-yellow calli were produced. The highest percentage (67.5%) of seed explants inducing callus was obtained on MS medium supplemented with 2.0mg.l⁻¹ of 2,4-D in the dark. When the calli were repeatedly subcultured in ½MS basal medium with a 16-h photoperiod without any hormonal supplement, adventitious shoots were produced eight weeks after culture initiation. The shoots rooted two weeks later. This is the first protocol of *in vitro* regeneration described for *A. arctotoides* and will serve as a foundation for further research on tissue culture, micropropagation and germplasm conservation of this important medicinal plant.

P2594. Employment of *in vitro* morphogenesis of onion for the breeding purposes

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This work describes an effective method for plant regeneration of onion through *in vitro* culture of ovary and flower explants. The presence of 2,4-D or kin in culture medium is required to induce acceptable yield of gynogenic cultures from immature flower or unfertilized ovary of onion. The highest frequency of *in vitro* differentiation of the two types of explants was registered with 2 mg /l 2,4-D + 1 mg /l kin containing medium. Incorporation of culture medium with 60 mg /l adenine sulfate obviously enhanced the proliferation capacity and growth characters. Among three concentrations of IBA and NAA used for rooting, the highest percentage of root formation as well as number of roots / shoot were noticed with medium contained 2 mg /l NAA. The chromosome count indicated that fifty percent of plantlets regenerated from ovaries were haploid (8 chromosomes). However, 33 % of flower-derived plantlets were haploid. The obtained plantlets were successfully adapted to free-living conditions after phase of acclimatization.

Key words : Onion - Haploid - *In vitro* - Gynogenesis .**P2595. Induction of gynogenic haploids in mulberry**

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Gynogenic haploid plants have been obtained from unpollinated ovules of mulberry variety-S₅₄ when cultured on half strength LSBM supplemented with 8% sucrose and BAP (2 mg l⁻¹). After 8 weeks of culture, shoot buds were initiated, which were transferred to full strength LSBM supplemented with BAP (2 mg l⁻¹) to obtain multiple shoots. These multiple shoots upon subculture developed roots on full strength LSBM fortified with BAP (2 mg l⁻¹) and NAA (1 mg l⁻¹). The well developed complete plantlets were hardened and established in soil with 78% survival frequency. The acclimatized plants retrieved from unpollinated ovule culture were screened cytologically adopting root tip squash technique, which revealed haploid number of chromosomes n=14 in their root tips. Further confirmation of ploidy level was made in these gynogenic haploid plants by adopting chloroplast count technique, which showed the presence of 8 chloroplasts in two guard cells of the stomata.

P2596. Callus Induction and Plant Regeneration From Mature Embryos of Different Wheat Genotypes

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Mature embryos of five *T. aestivum* and five *T. durum* cultivars formed embryogenic callus on two different media. Embryos were removed from surface sterilised seeds and placed with the scutellum upwards on a solid agar medium containing the inorganic components of Murashige Skoog and 2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) or 1 mg/L naphthalenacetic acid (NAA). The developed calli and regenerated plants were maintained on 2,4-D or NAA free MS medium. Wheat plants can

be regenerated via two different systems. There were significant differences in percentage of callus induction and regeneration capacity on the different initiation medium. Among the *T.aestivum* cultivars, Yakar had the highest regeneration capacity in both induction medium. In *T.durum* cultivars, Kiziltan gave the highest regeneration capacity in MS+2,4 D medium and Yilmaz gave the highest regeneration capacity in MS+NAA medium. A strong genotypic effect on the culture responses was found for both induction medium.

P2597. In vitro regeneration and field performance of *Tylophora indica* (Murm. f.) Merrill. - an endangered medicinal plant

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A protocol has been developed for high frequency shoot regeneration and plant establishment of *Tylophora indica* from petiole derived callus. Optimal callus was developed from petiole explants on Murashige and Skoog (MS) basal medium supplemented with 10 μ M 2,4-D + 2.5 μ M TDZ. Adventitious shoot induction was achieved from the surface of the callus after transferring onto shoot induction medium. The highest rate (90%) of shoot multiplication was achieved on MS medium containing 2.5 μ M TDZ. Individual elongated shoots were rooted best on half-strength MS medium containing 0.5 μ M IBA. When the basal cut ends of the *in vitro* regenerated shoots were dipped in 150 μ M IBA for half an hour followed by transplantation in plastic pots containing sterile vermiculite, a mean of 4.1 roots per shoot were developed. The *in vitro* raised plantlets were successfully established in earthen pots containing normal garden soil and grown in green house with 100% survival. After four months of transfer to pots, the performance of *in vitro* raised plants was evaluated on the basis of selected morphological and physiological parameters and compared with *ex vitro* plants of the same age.

P2598. In Vitro propagation of *Echinodorus ozelot* by multiple shoots formation.

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Echinodorus ozelot is a tropical aquatic plant which is in the family of Alismataceae. This fresh water plant is a hybrid between *E. schlueteri* and *E. barthii*. It has red brown leaves with black spots so it is very popular for decorating the fish aquarium. In nature, propagation of *E. ozelot* is a slow process so tissue culture techniques are an alternative means of plant vegetative propagation in short time and space. In this work, *E. ozelot* rhizomes were surface sterilized and grown *in vitro* in liquid basal medium of Murashige and Skoog (MS) supplemented with different combinations of naphthaleneacetic acid (NAA) and benzylamino purine (BAP). No multiple shoots of *E. ozelot* were induced in the medium without BAP and increasing BAP concentration increased the number of shoot formation. Increasing NAA concentration in the medium decreased the number of shoot formation but induced the root formation. After they produced roots, they were successfully established in the aquarium.

P2599. Somaclonal variation in *Zingiber officinale* : Screening for tolerance to *Pythium aphanidermatum*

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Somaclonal variants tolerant to rhizome rot disease have been isolated through callus phase using *in vitro* pseudostem and sheathing leaf base explants derived from direct regeneration of *in vivo* vegetative bud of *Zingiber officinale* Rosc. Profuse, compact, creamish white callus was obtained on LSBM fortified with 2,4-D (3 mg l⁻¹), which differentiated into complete plantlets on LSBM fortified with BAP (4 mg l⁻¹). The retrieved 982 plants were screened at culture conditions and 58 morphological variants were isolated. These somaclones were subjected to *ex vitro* sick plot technique to evaluate their disease tolerance potential. The survived somaclones were established in soil for rhizome formation (V₁ generation). The seed rhizomes were subjected to *in vivo* sick plot technique to confirm their disease tolerance potential during V₂-V₄ generations and their tolerance:susceptible ratio was recorded. The tolerant somaclones were evaluated for quality attributes, which were found to be superior and breeding true for

all the traits in successive V₂-V₄ generations suggesting their distinct fidelity over the parental plants.

P2600. Isolation of tolerant clones to culture filtrate of *Pythium graminicolum* through *in vitro* selection technique

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Root rot disease tolerant clones of turmeric variety Suguna were isolated using continuous *in vitro* selection technique against pure culture filtrate of *Pythium graminicolum*. Profuse, compact, creamish white callus obtained from *in vivo* vegetative bud was challenged with pure culture filtrate of *P. graminicolum* to isolate survivable callus, which was further subjected to pure culture filtrate treatment. After three cycles of treatment, four cell lines CL1a₁ and CL1a₂, CL1b, CL2 tolerant to 1% and 2% culture filtrate respectively was isolated through continuous *in vitro* selection and subcultured on non-culture filtrate regeneration medium along with the non-selected callus to obtain complete plantlets through discontinuous *in vitro* selection technique. Plants from tolerant and non-selected calli were subjected to *in vitro* sick plot technique and screened for disease tolerance, which revealed a ratio of 225:49 tolerant:susceptible clones. However, plants from CL1a₁ and non-selected callus were susceptible under *in vitro* sick plot technique. The disease tolerant clones were hardened and established in soil with 90% survival frequency.

P2601. Genetic and Phenotypic Characterization of "TPS1" Transgenic Rice Plants

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We obtained the 4 T₂ lines transformed with TPS1 gene and assessed the phenotype and elucidated the inheritance of the transgene using advanced generations. Bar gene analysis revealed that no segregation was observed in T₄ generation, indicating that the transgenic lines were homozygous. A possible explanation for the segregation data obtained from the transformed line and its descendants demonstrated that the T-DNA insertion locus was stably inherited and the linked 2 copies of the T-DNA insert from TPS1 were incorporated into the rice genome. Drought tolerance of transgenic plants was tested through the treatment of 10% and 20% PEG. The TPS1 transgenic rice plants show no significant resistance to PEG stress compare to wild type plants due to the fact that TPS1 rice plants produced only a trace of trehalose during PEG stress. This suggests that TPS1 gene is not effective enough to protect transgenic rice plants against stress.

P2602. Isolation and characterization of microsatellites loci in the lemon (*Citrus limon*)

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Microsatellite loci are highly informative genetic markers useful for population genetic studies, linkage mapping and parentage determination. A major limitation of SSRs is the time and cost required to isolate and characterize each locus when pre-existing DNA sequence is not available. In *Citrus*, enough sequence information is not available in database to design primers for microsatellite analysis. This study reports the isolation and characterization of seven polymorphic microsatellite loci in *Citrus*. The loci were isolated from two libraries constructed from genomic DNA nonenriched for TC and AC and enriched for AC repeats. These markers yielded 4 to 9 alleles per locus (mean 6.14) in a survey of 32 *Citrus* cultivars. Average observed heterozygosity ranged from 43 to 72% and expected heterozygosity from 57 to 78%. The levels of polymorphism found in this study suggest that these microsatellite loci can become an important tool for genetic studies in *Citrus*.

P2603. The wild species of the genus Brassica, Cucumis and Solanum in somatic hybridization

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The aim of the study is using wild species of the *Solanaceae*, *Cucurbitaceae* and *Brassicaceae* in somatic hybridization. The effort is introduction of resistance genes to various diseases from wild plant species to cultural crops. Protoplasts (mesophyll, hypocotyl, callus) were isolated from *in vitro* plants of *Armoracia rusticana*, *Brassica carinata*, *B. rapa*, *B. napus*, *B. oleracea*, *Cucumis sativus*, *C. metuliferus*, *C. melo*, *Solanum tuberosum*, *S. berthaultii*, *S. bulbocastanum*, *S. pinnatisectum* and *S. verrucosum*. Protoplast electrofusion was performed in chamber with 0,5 mm electrode distance. These parameters were tested: for protoplast alignment 4 - 10 V AC and 1 MHz frequency with duration of action 1 - 18 s, for fusion 1 pulse 10; 20 or 30 V DC of length 80; 40 or 30 μ s. Regeneration of cell walls and first division was recorded in most cases. In *Solanum* genus microcolonies were found in 15 days, start of plant regeneration in 140 days. In this case 75 plants were obtained after fusion in four combinations. This study was supported by project MSMT CZ OC 843.20, MSM6010980701 and NAZV MZe CZ QF 4108, QF 4133.

P2604. Efficient co-transformation and segregation of uncoupled T-DNAs transferred to barley (*Hordeum vulgare* L.) by a combination of different *Agrobacterium tumefaciens* strains

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Co-transformation techniques are required for independent integration of effector and selectable marker genes, or for the stacking of multiple transgenes. The removal of selectable marker genes (e.g. via segregation) is often necessary prior to transformation with another effector gene or cultivation of transgenic plants in the field. The need for the uncoupled integration of transgenes, along with our previous finding that barley can be efficiently transformed by *Agrobacterium* strain LBA4404 as well as by AGL1, led us to pursue a co-transformation approach using different *Agrobacterium* strains for the expression cassettes to be independently integrated into the barley genome. In this study we introduced a binary vector harbouring the selectable marker gene *HPT*, coupled with the *GFP* gene, into LBA4404 and another binary vector carrying the *GUS* gene without a selectable marker into either LBA4404 or AGL1. All employed transgenes were driven by appropriate constitutive promoters. The results obtained with regard to transformation, co-transformation, integrated copy numbers and segregation of the co-transformed T-DNAs in the progeny will be presented.

P2605. High frequency regeneration of *Gladiolus* through *in vitro* culture of nodal segments of scape and petal derived callus

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Direct shoot bud induction and regeneration of plants were achieved through *in vitro* culture of nodal segments of scape of gladiolus. Nodal segments cultured in MS medium supplemented with 1.5 mg/l BA + 0.5 mg/l NAA induced shoots in 80-85% of cultures within three weeks with 10-12 shoots per culture. Addition of 15% coconut water to the medium increased the number of shoots up to 20 per culture. The shoots were rooted in half strength of MS medium with 2.0 mg/l IBA and 6% sucrose. Callus capable of plant regeneration was initiated at high frequency from flower petals cultured in MS medium supplemented with 2.0 mg/l BAP or 1.0 mg/l 2,4-D. Callus was maintained in callus initiation medium. Shoots were regenerated from the callus in MS medium with 0.5- 2.0 mg/l Kn or/and 0.5-2.0 mg/l BAP. Highest number of shoots was regenerated in MS medium with 2.0 mg/l BAP from one-month-old callus. Shoot regeneration potential of callus decreased with the age and after fifth month no shoot was found to be regenerated. The shoots produced roots in half strength of MS medium with 2.0 mg/l IBA and 6% sucrose. Rooted shoots of both the categories produced cormels at the base of shoots in the same nutrient medium. About 80-90% cormels germinated when sown in the field and 20% of them blossomed in the first year.

P2606. Somatic embryogenesis in a transformed cell culture of *panax ginseng*

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Expression of the *Agrobacterium rhizogenes rolC* gene in *Panax ginseng* callus cells results in formation of tumors that are capable to form roots. The selection of the tumor clusters, which did not develop roots, yielded the embryogenic 2c3 callus line. The ability to form somatic embryos is independent of external growth factors. Such developmental shift is of particular interest because this is the first indication of the *rolC*-embryogenic effect and, to our knowledge, the first indication that a single gene of non-plant origin can induce somatic embryogenesis in plants. The DNA sequence analysis showed that the somatic-to-embryonic transition was not due to a mutation of the *rolC* gene inserted. Although the 2c3 somatic embryos developed through a typical embryogenesis process, they terminated prematurely and repeatedly formed adventitious shoot meristems and embryo-like structures. A part of the shoots and somatic embryos formed dramatically enlarged and fasciated meristems. The observed developmental abnormalities are similar to those that have been previously described for *Arabidopsis* embryogenic calluses where the *WUSCHEL* gene was continuously overexpressed.

P2606a. Determination of The Microspore Development Stage of Different Sized-Buds in *Asparagus (Asparagus officinalis* var. *altilis* L.) for Anther Culture

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A series of factors such as genotypes, explants, culture media, pre-treatments and incubation conditions affect the results of *in vitro* anther culture. One of these factors is microspore development stage when flower bud is excised from plant. For most species, the *in vitro* anther culture gives the best results when microspores are at the uninucleate stage or right after this period. In order to determine the optimal stage for androgenesis, male buds were divided into three groups according to their length and width (The first group: 2,5- 2 mm; the second group: 3,6- 2,5 mm and the third group: 4.0-2.5 mm). Anthers were stained with ethidium bromide (3,8-diamino-5-ethyl-6-phenyl phenanthridium bromide) and examined in fluorescence microscope. At the end of the study it was observed that pollen from 4 mm length and 2.5 mm width flower buds were binucleate stage, while the others were uninucleate stage. The results revealed that this staining method could be prepared easily and its results could be observed immediately without any pre-treatments.

P2607. Produce of functional plants introduced the CD98 gene for tumor-specific protein

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In an attempt to produce physiologically functional plants exhibiting an anti-cancer effect, we prepared transgenic plants transformed with the CD98 gene for a tumor-specific, membrane-located protein. The CD98 protein plays a significant role on amino acid transport and integrin-mediated adhesion, and is strongly produced in tumorous cells of kidney, small intestine, testis and ovary. In this study, the gene of CD98 taken out of the cancer cell was inserted in binary vector (pIG121-hm), and was introduced into *Louis japonicus* by using an *Agrobacterium*-mediated method. The expression of CD98 gene in plant was detected by RT-PCR and Western blotting analysis. As a result, hygromycin-resistance lines selected were shown to successfully transcribe the introduced gene and translate the CD98 protein. Thus, the present study suggests that our approach provides an experimental basis for production of edible vaccine against CD98.

P2608. Metabolic engineering of flavonoid biosynthesis to modify flower colour-from blue to pink and yellow

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Flavonoids confer various flower colours such as yellow (chalcones and aurones), orange/red (typically pelargonidin), red/magenta (cyanidin) and violet/blue (delphinidin).

Torenia hybrida cv. Summerwave Blue and Violet mainly produce delphinidin. Down regulation of their respective flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase (F3'5'H) genes accompanied by over expression of rose or pelargonium dihydroflavonol 4-reductase (DFR) yielded pelargonidin-based bright pink flowers. Expression of snapdragon chalcone 4'-glucosyltransferase and aureusidin synthase genes and down regulation of anthocyanin biosynthesis produced aureusidin based yellow colour.

Nierembergia cv. Fairybells also lack pink flowers as they produce delphinidin and flavonols. Pelargonidin-based pink flowers were generated by down regulation of F3'5'H and flavonol synthase genes accompanying over expression of rose DFR. These results indicate that engineering the flavonoid biosynthetic pathway is a powerful way to achieve novel flower colours.

P2609. Light quality influences indigo precursors production and seed germination in *Isatis tinctoria* L. and *Isatis indigotica* Fort.

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Isatis tinctoria L. and *Isatis indigotica* Fort are biennial herbaceous plants belonging to the family of Cruciferae that are used as a source of natural indigo and show several morphological and genetic differences. Production of Indigo (indigotin) precursors, Indican (indoxyl β-D glucoside) and Isatan B (indoxyl ketogluconate), together with seed germination ability were compared in *Isatis tinctoria* and *Isatis indigotica* grown under six different light conditions (darkness, white, red, far red, blue, yellow light) at 25 °C. Light quality influenced both germination and production of indigo precursors in the two *Isatis* species suggesting a different role played by the labile and stable phytocrome species (phyA and phyB) in the Isatan B production in *I. tinctoria* and *I. indigotica*.

Our results provide further insights on the distinct behaviour of *I. tinctoria* and *I. indigotica* that belong to two different genetic clusters and different original environments.

P2610. Associations between phenotype variation and gene alteration in induced mutations.

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Our research employs gamma radiation to induce genetic change in economically important species from diverse taxa. The association between genetic change at the DNA level and specific phenotypic variants is being investigated. Our work aims to correlate the expression of altered grain quality characteristics in induced mutants with specific genetic changes at the molecular level, using gene-specific PCR primers targeting food quality trait genes. The objective is to better understand the relationships between certain DNA sequences and grain quality attributes amongst our gamma mutated crop populations, which include mung beans and sorghum. For genes associated with various grain quality traits, a high resolution capillary electrophoresis system is used to identify regions which have altered DNA composition. Sequencing of the altered gene regions in mutants exhibiting changed grain quality characteristics is shedding light on the genetic determinants of grain flavour and starch properties.

P2611. Towards generation of plant N-glycans containing biantennary 1,4 galactose

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Plants are attractive hosts for the production of recombinant proteins of pharmaceutical interest. The majority of therapeutically relevant human proteins need N-glycosylation if to retain full activity. Plant derived glycoproteins contain N-glycans with (1,2)-linked xylose and core (1,3)-linked fucose residues, which are not present in humans, on the other side plant N-glycans lack terminal 1,4 galactose.

In a first approach triple knockout *A. thaliana* plants were generated, with complete deficiency of active (1,2)-xylosyltransferase and of core (1,3)-fucosyltransferase (XT/FT). These plants synthesise complex N-glycans that completely lack xylose and fucose. To elongate these structures by galactose corresponding mammalian enzyme, 1,4 galactosyltransferase (GalT) were transferred into XT/FT lines. For generation of desired

biantennary 1,4 galactose structures a series of fusion constructs containing the catalytic domain of GalT fused to different Golgi retention sequences was generated and transferred *A. thaliana* plants.

P2612. Effects of Over-expression of a Maize Bundle Sheath-specific Phosphoenolpyruvate Carboxykinase in Transgenic Tobacco on its Growth and Metabolism

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PEP carboxykinase (PCK) serves as a decarboxylation enzyme of oxaloacetate in the bundle-sheath cells in PCK type C4 plants. For introduction of a PCK-type C4-like pathway into C3 plants, a maize bundle sheath-specific PCK gene was over-expressed in tobacco under the control of tomato *rbcS-3C* promoter with its transit peptide. Western analyses showed that the expressed PCK protein was localized in the chloroplasts of the transgenic tobacco. Unexpectedly a single introduction of PCK enhanced the photosynthetic activity and the phenylpropanoid synthesis such as chlorogenic acid. The transgenic tobacco had high level of chlorophyll in their mature leaves. The growth of the transgenic plants was increased significantly when they were grown in a growth chamber under normal conditions and enhanced more significantly under adverse conditions. The growth of the transgenic tobacco showed some correlation with PCK protein level in the plants. The increases in the contents of starch, sucrose, glucose and fructose and aromatic amino acids were also observed in the mature leaf of the transgenic tobacco.

P2613. Metabolic engineering of dhurrin biosynthesis in transgenic *Arabidopsis* plants with marginal inadvertent effects on the metabolome and transcriptome.

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Focused and non-targeted approaches were used to assess the impact associated with introduction of new high flux pathways in *Arabidopsis thaliana* by genetic engineering. *A. thaliana* plants expressing the entire biosynthetic pathway for the tyrosine derived cyanogenic glucoside dhurrin as accomplished by insertion of *CYP79A1*, *CYP71E1*, and *UGT85B1* from *Sorghum bicolor* accumulated 4% dry-weight dhurrin with marginal inadvertent effects on plant morphology, free amino acid pools, transcriptome and metabolome. In a similar manner, plants expressing only *CYP79A1* accumulated 3% dry-weight of the tyrosine derived glucosinolate, *p*-hydroxybenzylglucosinolate with no morphological pleiotropic effects. In contrast, insertion of *CYP79A1* plus *CYP71E1* resulted in stunted plants, transcriptome and metabolome alterations. The pleiotropic effects observed in plants expressing sorghum *CYP79A1* and *CYP71E1* were complemented by retransformation with *S. bicolor UGT85B1*. Accordingly, high flux pathways directing synthesis and intracellular storage of high amounts of natural products is achievable in transgenic plants with marginal inadvertent effects.

P2614. A functional genomics approach to unravel plant secondary metabolism in *Catharanthus roseus* by combining transcriptional profiling with metabolomics

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The medicinal plant *Catharanthus roseus* has been extensively investigated during the last decades in order to utilize cell culture systems for the biotechnological production of important anticancer compounds e.g. vinblastine and vincristine. Despite considerable progress on the genetic and enzymatic level, especially the very complex regulation of the biosynthetic pathway is still poorly understood. We have applied a comprehensive profiling approach based on functional genomics, which is independent of prior sequence knowledge, to monitor jasmonate-induced changes on

the transcript and metabolite profiles of cell cultures. cDNA-AFLP based transcript profiling allowed the visualization of more than 10000 gene tags, more than 500 of which were differentially regulated, and include not only structural but also regulatory genes i.e. transcription factors. Additionally, gene expression could be linked to LC-MS based metabolomics, comprising hundreds of compounds, and targeted metabolite analysis, leading one step further towards 'Systems Biology'.

P2615. Effect of salicylic acid on yield, yield components, anatomical structures and some biochemical aspects of chickpea (*Cicer arietinum* L.)

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Salicylic acid (SA) is now considered as a hormone like substances which affects on plant growth and development.

A field experiment was conducted during 2004 in Shahreray Azad university to determine the effects of SA on yield, yield component and anatomical structure of chickpea cultivar Bivanij.

The experimental design was a split plot in RCBD with 4 replications in which main plot were 2 treatment methods (spraying and watering SA) and subplot were 4 concentrations of SA (0, 0.1, 0.7, 1.5 mM).plant treatment started at beginning of flowering during 40 days.

The results showed that spraying SA was better than watering.

Yield, 100-seed weight, 100-pod weight, secondary branches, plant height, total soluble proteins were maximum when 0.7 mM SA was sprayed.

The number of stomata increased when 0.1 mM SA sprayed. 1.5 mM SA damaged parenchyma tissues in leaves when it watered, as well as sclerenchyma tissue in stems, xylem in root and the number of leaves trichomes were increased.

The patterns of peroxidase isoforms resulting from leaves were determined by means of polyacrylamid gel electrophoresis. One new lightly band was appeared when 1.5 mM SA was sprayed.

P2616. Genetic modification of soybean (*Glycine max*) - tocopherol using tocopherol methyltransferase gene via *A. rhizogenes* transformation

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Soybean genetic modification was performed in order to alter the vitamin E composition aimed at understanding the good food quality as a change -tocopherol to -tocopherol. Vitamin E, which is composed of the tocopherol is a lipid-soluble antioxidant and -tocopherol has the highest vitamin E activity compared with other type of tocopherol. Meanwhile, a lot of oil plants contain more -tocopherol than -tocopherol. In order to convert -tocopherol to -tocopherol in soybean, we have cloned -tocopherol methyltransferase (*tmt*) gene from *Perilla frutescens*, constructed binary vector harboring *bar* and *tmt* gene and transformed soybean by *A. rhizogenes* mediated method. Putative individual transformed hairy roots were screened for transgene incorporation using PCR and genomic Southern blot analysis after *agrobacterium* decontamination. The expression of introduced-*tmt* gene was confirmed in hairy roots of soybean by Northern blot. As the tocopherol composition in the transgenic soybean hairy root was analyzed by HPLC, the data showed that more than 90% of -tocopherol in the transgenics was converted into -tocopherol.

P2617. Brassinolide improves the physiological efficiency in growth and seed yield of black gram (*Vigna mungo*)

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The imbalanced relationship between the source and sink organs causes a considerable reduction in the potential seed yield of black gram. Early leaf senescence, excessive flower drop and poor harvest index are the factors that cause less productivity. Studies were undertaken in black gram cv. ADT 5 to tap its potential yield through physiological alterations in the plant system. Naphthalene acetic acid (NAA), salicylic acid (SA), mepiquat chloride (MC), brassinolide (BR), triacontanol and a micronutrient formulation were fed to the plants as foliar application at pre-flowering stage.

NAA 40 ppm caused increased plant height, more number of branches, higher leaf area index and leaf area duration. But, with regard to physiological parameters, the photosynthetic efficiency was observed high in BR 0.1ppm treated plants as evidenced by the higher specific leaf weight, chlorophyll, soluble protein, stomatal conductance and nitrate reductase activity. Among the treatments, BR 0.1ppm resulted in higher dry matter accumulation, more number of pods and seeds per plant and thereby maximum seed yield. Seed protein content was also found improved due to BR application.

P2618. Production of isoflavone in *IFS1* transgenic rice

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Isoflavonoids are a diverse group of plant natural products synthesized from phenylpropanoid which play important roles in plant growth and development, and in defense against microorganisms and pests. These compounds often possess antioxidant and estrogenic anti-cancer activity, and are also receiving considerable attention as health-promoting nutraceuticals. Metabolic engineering for production of isoflavone in rice may provide the health benefits of these phytoestrogens from consumption of more widely used grains.

We cloned *IFS1* from Korean soybean cultivar, Sinpaldalkong 2 known as one of isoflavone rich cultivars with PCR and specific primers. Expression vector of *Glb-SinIFS1* for rice seed specific expression was constructed and transferred to binary vector. The plasmid was introduced into color rice containing flavanones. Expression of *Glb-SinIFS1* gene in the color rice resulted in the synthesis and accumulation of the isoflavone genistein in seeds. This study provides a good example for development of new trait rice added health promoting nutraceuticals through the genetic manipulation.

P2619. Engineering wheat with improved resistance to Fusarium head blight

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Fusarium head blight or scab is one of the most destructive fungal diseases of wheat, causing significant reductions in grain yield and quality. Trichothecene mycotoxins (e.g. deoxynivalenol or DON) produced during infection further reduce grain quality. Our goal is to improve host resistance to scab by introducing genes whose products might induce wheat defense responses or decrease the toxicity of trichothecenes. In order to limit initial fungal infection, wheat was transformed with genes controlled by the organ specific promoter, *Lem1*, and encoding enzymes involved in activated oxygen metabolism. The introduced enzymes accumulate in the outer floral organs from anthesis to early kernel development, during which period wheat is most vulnerable to *Fusarium* infection. Mitigating trichothecene toxicity was achieved by introducing genes encoding DON acetyltransferase and a trichothecene efflux pump. Their constitutive co-expression results in a 40-50% reduction in the spread of *Fusarium* infection through the spike. The synergistic effects of the transgenes on enhancing plant structural defenses and decreasing DON levels will be discussed.

P2620. A strong non-specific upstream regulatory region for gene expression in plants

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The two versions of the upstream transcription regulatory regions of a peanut gene differing by the presence of an intron were translationally fused to a reporter gene GUS and the resulting cassettes were stably introduced in tobacco by *Agrobacterium*-mediated transformation. The intron-containing construct resulted in plants with the moderate levels of the GUS activity whereas the construct without introns was about 10-fold more active in all plants analysed. The GUS activity measured in leaf extracts of some of the latter plants was about two- to three times higher than the activity usually obtained from the CaMV 35S promoter. These results together with our earlier transient expression experiments

on this regulatory region support the hypothesis that the intron in question contains a tissue-specific transcriptional silencer and can be used to genetically engineer peanut in order to control its contamination with aflatoxin and for the other agriculturally important traits. The strong promoter variant may certainly be used for the overexpression of the genes of interest.

P2621. Development of acyanogenic cassava lines by RNAi technology in African elite varieties

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Cassava, *Manihot esculenta*, is the most important root crop and the third most important staple food in the Tropics. Major deficits of cassava are low protein content in the tubers, fast post-harvest tuber deterioration and high content of the two cyanogenic glucosides linamarin and lotaustralin. Upon tissue disruption, the cyanogenic glucosides are degraded resulting in release of hydrogen cyanide. No acyanogenic cassava cultivars are known. Use of cassava as a staple food requires processing to remove the cyanide. Proper processing tends to result in loss of protein, minerals, and vitamins.

Cassava is allopolyploid and we have isolated the two *CYP79* paralogs, *CYP79D1* and *CYP79D2*, which encode enzymes catalysing the rate limiting step in cyanogenic glucoside biosynthesis. To block this pathway, RNAi technology has been used with a construct containing a 300 bp fragment of each of the *CYP79* paralogs.

Using RNAi technology we have produced acyanogenic cassava lines from several African elite lines. Analysis of the leaves of transformants revealed lines in which the cyanogenic glucoside content was reduced to less than 1% of wild-type levels.

P2622. Effect of anthocyanins, extracted from berries cultures, on cancer cells

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Anthocyanins, present in fruits and vegetables, belong to phenolic phytochemicals linked to reducing the risk of cancer.

This work has been undertaken in order to investigate the effect of anthocyanins, produced by berries cultures, on proliferation of B16-F10 melanoma murine cell line.

Anthocyanins were extracted from suspension cultures of strawberry (*Fragaria x ananassa* Duch.), raspberry (*Rubus idaeus* L.), and blackberry (*Rubus fruticosus* L.). To increase pigments production, the cultures have been exposed to blue light (400-600 nm) or red light (675 nm); control cultures were exposed to visible light. At the end of the experiments, anthocyanins were extracted and their amounts were determined by spectrophotometer.

The growth of the cultures was enhanced by different lights only in raspberry (blue and red light) and blackberry (blue light), while anthocyanin yield was not affected by the type of light.

The higher inhibition of melanoma cell growth was obtained by treating the cells with anthocyanins extracted from berries cultures exposed to blue light.

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P2623. Medicinal plants used by Traditional Birth Attendants in Kenya

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Maternal health provision in Kenya is beyond the reach of most rural women populations. Medicinal plants in Kenya are widely used by TBAs in maternal and child healthcare management. Ethnobotanical surveys were carried out to document medicinal plants used in traditional midwifery in Kamba community of Kenya. The study shows that medicinal plants are widely used by traditional midwives during pregnancy, delivery and during post-natal period. The high use of medicinal plants is mainly due to poor road net network, scarcity of health facilities and unaffordable modern medicine. During the study, 430 respondents (TBAs) were interviewed within four districts which form the Kamba community

in Eastern province of Kenya. Two hundred plant species were found to be used at one point of the maternity circle or another. Consensus as a quantitative criterion was used in recording field information. Descriptive statistics was used in data analysis. Population reduction criterion was used to determine priority species in need of urgent conservation. Based on this, 14 plant species were found to have become rare due to over exploitation.

P2624. Physiological responses of parsley plants (*Petroselinum crispum*) to Cr⁺³ accumulation

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Cr⁺³ is an essential micronutrient for carbohydrate and lipid metabolism in human and animals. One of the ways for preventing of chromium deficiency and its undesirable effects can be using chromium accumulator plants in daily diets.

For this purpose, parsley (*Petroselinum crispum*) seedlings were grown at hydroponic culture (Hoagland) containing various levels of Cr⁺³ (0.1- 3 mg/l). After 5 weeks, Cr⁺³ accumulation and its effects on growth rate (root and shoot dry weight and length), chlorophyll a and b content and concentrations of Ca⁺², K⁺ and Na⁺ in the roots and shoots were studied.

Results showed that parsley growth, chlorophyll content and concentrations of Ca⁺², K⁺ and Na⁺ decreased as Cr⁺³ concentrations increased in growing media.

With increasing Cr⁺³ levels in medium, its concentration in the roots and shoots increased significantly, so that in plants grown at tolerance threshold concentration (2 mg/lCr⁺³), chromium content in the root and shoot was 22 and 6 fold higher than the plants grown at 0.1 mg/lCr⁺³, respectively. Chromium accumulation in the root was more than the shoots.

P2625. Changes in morphine alkaloid pattern of *papaver somniferum* seeds as a result of thermal degradation

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The content of opium alkaloids in *papaver somniferum* seeds varies between 2 and >964 mg/kg for morphine, 0.1 and 79 for codeine and 5 and 30 for thebaine. The level of total opium alkaloids in blue poppy seeds is tentimes the quantity in grey sorts. Containing higher amounts of alkaloids there is no great demand in consequence of their pharmacological-toxicological properties and their possibility of improper use. Only a few grams of this natural product and someone can be suspected of having consumed drugs. Extensive studies were necessary to find out the perfect temperature of 140°C during 45 minutes to reduce the alkaloid content of poppy significantly. In the case of blue poppy seeds morphine decreased from 270 to 180 mg/kg (65 % related to the initial state), codeine from 75 to 44 (60 %) and thebaine from 30 to 9 (30 %). The loss of thebaine can be explained with sublimation process, by morphine and codeine mainly a thermal induced formation of alkaloid condensation products has to be expected. It is a further advantage of thermal treatment of poppy seeds that microorganisms are destroyed and free fatty acids are lowered, thus obtaining a better food quality.

P2626. Aromatic plants for food industry - researches in Ukraine

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Intensive researches with aromatic plants in Ukraine began in the second part of XX c., and in department of new cultures of National Botanical Garden of NASU - since 1974. The main purpose was to substitute imported spice and used widely in perfumery industry and food enrichment of the cultivated flora medicinal. High price of exotic spice in our country has caused necessity to find spice plants in aboriginal flora of Ukraine or to introduce of them from the South. For the first time in Ukraine were assembled about 120 kinds aromatic plants. The origin most of them is tropics and subtropics. Our studies identified a number of aromatic plants, among them we selected edible plants from Mint Family (Lamiaceae): *Lophanthus*, *Monarda*, *Majorana*, *Salvia*, *Hyssopus* etc. which cultivated to be used as herbs and as a source of essential oils.

In the several last years most of researches on the aromatic plants were focused on the adaptability of the crop to local conditions.

Studies of the quality essential oil were conducted, to help evaluate the commercial potential of the crop in Ukraine. Essential oil was obtained by water distillation procedure and identification of components by GC.

P2627. Cornelian cherry (*Cornus mas* L.) as alternative species for utilization in diet, landscape architecture and pharmacy

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Cornelian cherry belongs among originally widespread species in Europe. It is potential economical valuable fruit species also for cultivation in monocultures. Experimentally were evaluated 250 ecotypes from the Gemer region in Slovakia. The fruit weight was in range 0.6 to 2.7g, fruit length 13.2 to 20.1 mm, proportion of the flesh 77 - 86%, content of vitamin C 16.45 - 78.58 mg/100g, content of organic acids 4.6 - 7.4%, and total amount of sugars 6.6 - 15.1%. Descriptor list for evaluation of genotypes was made on the results base. Experimental data and image documentation were used in processing of the information system for genotypes in GENOTYPDATA *Cornus mas* L. evidence. Results confirmed possibility of genotypes detection as well as selection for economical utilization.

P2628. The Effects of Aqueous and Alcoholic Extracts of Walnut's Leaves (*Juglans regia*) on the Blood's Biochemical Factors of Diabetic Rats

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In this investigation the effects of aqueous and alcoholic extracts administration of walnut's leaves (*Juglans regia*) on the serum levels of glucose, urea, triglyceride and cholesterol of diabetic rats were studied

Aqueous and alcoholic extracts of walnut's leaves were prepared by maceration technique and then their lethal doses were measured. From 48 male and female rats, 8 rats considered as control (non diabetic) and the others were made diabetic by subcutaneous injection of 120 mg/kg body weight Alloxan monohydrate.

Diabetic rats were divided into experimental groups 1 (with no treatment), 2 (treated with 0.5 g/kg body weight aqueous extract) and 3 (treated with 0.35 g/kg body weight of alcoholic extract). Blood samples were collected weekly for a period of four weeks and the serum levels of glucose, urea, triglyceride and cholesterol were measured. The results indicate that administration of both extracts may lower the serum levels of glucose and urea and may increase the serum levels of cholesterol and triglyceride.

P2629. Essential oils of some Lamiaceae species: powerful inhibitors of lipid peroxidation

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With the increasing environmental pressures for industry to use "clean technology" and to utilize sustainable sources of materials for manufacturing process, recent researches are concentrated on natural sources of antioxidants such as different plant extracts.

In this study, the effects of essential oils obtained from ten Lamiaceae species (genera *Melissa*, *Mentha*, *Ocimum*, *Origanum*, *Rosmarinus*, *Salvia* and *Thymus*) on Fe²⁺/ascorbate and Fe²⁺/H₂O₂ induced lipid peroxidation (LP) in the liposomes was evaluated by TBA-test.

All examined essential oils expressed strong inhibition of LP in both systems of induction. In the Fe²⁺/ascorbate induced LP all tested essential oils exhibited very strong influence on LP, amongst which highest inhibition was expressed by essential oils of *Mentha longifolia* (94%) and *Origanum vulgare* (85.46%). In the Fe²⁺/H₂O₂ induced LP the highest LP inhibition was observed with the oils of *Melissa officinalis* (94.59%) and *Salvia nemorosa* (84.69%). The obtained results confirm the standpoint that the plants of the Lamiaceae family are very promising sources of natural antioxidants depending to the essential oils constituents.

P2630. Studies in *Basilicum polystachyon* (L.) Moench: germination, micropropagation and production of antioxidants, caffeic acid and rosmarinic acid.

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The germination behavior of *Basilicum polystachyon* Moench, an ethnomedicinal herb, revealed that the seed dormancy could not be removed by physiological and scarification treatments. Treatment with 100ppm GA₃ resulted in 98% germination and it was found to be concentration dependent. Realising its rare occurrence, an efficient regeneration protocol was invented. To develop an *in vitro* regeneration method, the potentiality of various plant parts on Murashige and Skoog's medium (MS) supplemented with 0.5, 2.5, 5, 10µM naphthaleneacetic acid (NAA) and 6-benzyladenine (BA) either singly or in all possible combinations was studied. Green compact calli was obtained from the shoot tip explant within two weeks. Optimum concentration for multiple shoot regeneration was 2.5 and 10µM NAA and BA in combination producing 14.39±3.07 shoots. Rooting was achieved in half strength MS medium. The plantlets were transferred to the experimental plot after hardening with 99% survivability. *In vitro* flowering of rooted microshoots produced viable non-dormant seeds. Micropropagated plants revealed caffeic acid (0.804 mg/g f wt.) and rosmarinic acid (1.421 mg/g f wt.).

P2631. The Oily Pumpkin (*Cucurbita pepo* var. *styriaca*) as a source for nourishment and biological actively components

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In the experiments we have been evaluating a variability of 36 quantitative and qualitative characters on flowers, fruits and seeds of the oily pumpkin (*Cucurbita pepo* var. *styriaca*). We received the pumpkins from a vesture at acreage 30 ha. By the appreciation of the characters the following measures have been designated: seeds weight from fruit in a dry state 0.5 - 257 g, the number of evolved seeds in the fruit 8 - 800 pieces, a seed's width of 6.8 - 12.0 mm, a seed's length of 11.2 - 32.7 mm and a seed's thickness of 1.8 - 32.7 mm. We have found some correlation coefficient of r = 0.41 - 0.59, between the characters fruit length and seeds length, the fruit weight /seeds weight in fresh state with a correlation coefficient of r = 0.69 - 0.87 and the number of seeds in fruit/weight fruit with a correlation coefficient of r = 0.61 - 0.79. The important variability was found in the colour of the flesh and in the chemical composition of the flesh, the seeds and the flowers.

P2632. Effect of 40 Vegetable Extracts on Differentiation of 3T3-L1 Preadipocytes into Adipocytes

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Obesity, diabetes, hypercholesterolemia, and hypertension occur primarily genetically, but they are often triggered by imbalanced lifestyle related to overnutrition. This is the reason the diseases are collectively called "lifestyle related diseases", and as they are risk factors for atherosclerosis, their prevention is of great importance. The strategy is to take measures of primary prevention for lifetime. Diet rich in vegetable and fruit has been recommended to reduce the obesity and the risk of atherosclerosis, but their active components and mechanisms of action have not been fully understood. We examined 40 vegetable extracts for their effects on differentiation of 3T3-L1 preadipocyte cells to adipocytes. As the result, some inhibited lipid accumulation in the cells while the others accelerated it. We identified by HPLC analysis several phenolic compounds, which were partially responsible for the activity. Purification and characterization of other active ingredients are undergoing.

P2633. Citrus Flavonoids and Their Anticancer Activity

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Flavonoids are important secondary plant metabolites, and are mainly present in plant tissues in relatively high concentrations as sugar conjugates. Epidemiological studies have indicated that flavonoid consumption is associated with a reduced risk of cancer. The aim of this study was to survey the relationship between anticancer activity and the content of major flavonoid in the edible part of the representative Citrus species and the relatives according to Tanaka's classification. Various Citrus were subjected to screening for anticancer effect, which was assayed by the differentiating activity against promyelocytic leukemia cells, and to high-performance liquid chromatography analysis of flavonoids. We precisely evaluated the influence of cultivars on flavonoid composition by principal component analysis. The principal component analysis revealed that the distribution of Citrus species belonging to different classes was largely in accordance with Tanaka's classification system.

P2634. The effects of hydro alcoholic extract of soya in the histological structure and the level of estradiol and testosterone in male sorie

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Soja is a rich source of phytoestrogen. Therefore, the objective of this project was to determine whether soya extract can change the structure of male reproductive tissue and it can influence on the level of sex hormone. For this purpose, 50 male sories were selected and divided into 4 groups. 3 experimental groups were fed with 150, 100 and 75 mg/kg of hydro alcoholic extraction of soya and control group were fed with the water for 50 days. Blood samples were taken in zero and fiftieth day of experiment and the level of sex hormone were measured and reproductive tissue were removed, fixed, processed and stained with H and E staining method. Histological of sections were revealed some change in testis. In the testis many spermatocytes at the metaphase stage in 150 mg/kg and 100 mg/kg treated groups and some of these mitotic figures. In conclusion, it seems that soya extract can change the spermatocyte, in low concentration, it may induce proliferation and high concentration, it may lead to cell death and nucleus fragmentation. The result indicated that level of testosterone and estradiol was decreased significantly in the group that was fed with 150 mg/kg.

P2635. Changes in soya bean seed under unfavourable conditions

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Conservation of plant germplasm is important goal in plant breeding programs and in preservation of biodiversity. Fast germination decline during long-term storage may cause loss of important varieties. Changes in seed were investigated on four soya bean varieties. Two treatments of accelerated ageing method were used to produce the effect of prolonged storage. Standard germination test, cold test and tetrazolium test were conducted to determine the germination and vigour in seed. Soybean seed showed decrease in germination and vigorous seedlings number after AA treatments, especially in seed with lower initial germination. Protein content in soya bean seed with standard quality is 38 - 45 %, thus the seed has short storage life. The change of soluble protein and free amino acid content in the seed of investigated cultivars was determined. SDS-PAGE and densitometric analysis were used to determine the alterations of major soybean protein subunits as a function treatment conditions while the HPLC method was used to detect the change of sucrose content.

P2636. Antiproliferative and Antioxidant Properties of Crabapple Juices

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Juices prepared from 42 crabapples (*Malus* spp.) were studied attempting to explore their antiproliferative activity. Most of the juices examined showed strong inhibition on proliferation of HL-60

human leukemia cells. The amounts of total phenolics and total anthocyanin, as well as DPPH radical scavenging activity were measured. The antiproliferative effect was more correlated to the amount of polyphenol than that of anthocyanins. Generally speaking, the stronger DPPH radical scavenging activity, the higher antiproliferation on HL-60 was shown. The colors and sizes of fruit and the colors of flower were not strongly correlated to antiproliferative activity, although all of the middle size (1 to 3cm in diameter) fruits examined showed strong HL-60 antiproliferative and DPPH radical scavenging activities. The results suggested a possibility of utilizing crabapple juices as one of the processed foods and beverages contribute to keep good health.

P2637. Forest Tree Seeds: Alternative Source of Human Nutrition.

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Food the most important, fundamental need, evolved with the existence of the living world. Besides the edible seeds, which are consumed by the living world, forest tree seeds may be a good source of food. These seeds are available in abundance in the forest but are under utilized because of the ignorance of their nutritional status. Thus, 22 forest tree seeds from the Victoria Park (reserved forest) near Bhavnagar (Gujarat) were selected for the study and analysed for their protein, reducing and non-reducing sugars, starch and oil content.

From the estimation for biochemical constituents of seeds, out of 22 species 17 species (9 leguminous and 8 non - leguminous) had fairly good amount of protein. The seeds of 16 (10 leguminous and 6 non-leguminous) species found rich with the good amount of total sugar. Seeds of 14 species (9 leguminous and 5 non-leguminous) species found enriched with starch content. In case of oil content seeds of 10 non-leguminous species and seeds of 2 leguminous species had fairly good percentage of oil.

P2638. Effect of different pathogenic organisms on sugarcane juice quality

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Sugarcane diseases caused by pathogenic organisms have often been associated with juice quality changes. In this work, variations in some saccharimetric parameters as % of pol (% of sucrose by polarimetry), brix (soluble solids), reducing sugars, and purity, currently used as juice quality markers were analysed in response to different diseases.

To study Yellow Leaf Syndrome, a disease produced by a phytoplasma, two susceptible sugarcane cv. were chosen: C 120-78 and C 87-51. Results show that YLS infection results in high values of % of pol, brix and purity and low values of reducing compounds, which reflect a high juice quality.

To study leaf scald disease produced by *X. albilineans*, L 55-5 and C 439-52 cv., both susceptible to the disease were chosen. Results show that sugarcane juices from diseased plants presented lower pol, brix and purity values and higher reducing sugars concentration than those obtained from healthy plants.

Smut disease, caused by *U. scitaminea* was studied in two cv., My 55-14 (resistant) and Ba 42-231 (susceptible). Not significant differences in saccharimetric parameters were observed between healthy and diseased plants.

P2639. Optimisation of Flavonoid-rich Extracts of *Anthemis tinctoria* and their Applicability in Food Industry

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Anthemis tinctoria (Compositae) is a herbaceous plant widespread in Europe and formally used as natural dye for cloth.

Among the metabolic products are carotinoids and flavonoids which account for the brilliant yellow colours but as well protect cells and tissues by deactivating singlet oxygen. In this context, it is noteworthy to discuss *Anthemis tinctoria* as potential source for naturally occurring antioxidants in food systems.

The objectives were (1) to define optimum conditions for extraction in respect to antioxidant activity, total yield, total phenolic content and (2) to test the applicability of extracts in food technology. Optimum extraction conditions were found in the range of 40% to 60% ethanol, possessing antioxidant activity, shown by inhibitory

activity in sunflower oil oxidation (Peroxide value) and radical scavenging activity (DPPH° radical). Further research addressed various aspects of extracts obtained in pilot scale as useful food additive (colorant, antioxidant, preservative).

P2640. Identification and Characterization Of A Novel Biodegradable Polymer From Natural Cocos Kernel for its Pharmaceutical Application

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The main aim of our present investigation deals with the isolation and characterization of a stabilizer from *Cocos nucifera* kernel by elemental analysis, UV, IR, NMR and mass spectroscopic studies. Further, the isolated Cocos polymer was employed for the formulation of magnesium carbonate suspensions, aspirin granules for formulation of tablets and filmability character of the polymer was also assessed. The formulated suspensions were evaluated for its stability. The results revealed that the formulated suspensions showed promising stability. The aspirin tablets were formulated using Cocos polymer solution as binding agent. It was found that the Cocos polymer exerted good bindability. Cocos polymer also showed good filmability, which was confirmed by evaluating the casted films.

Finally, the conclusions were drawn that the novel Cocos stabilizer is polymeric protein in nature and it can be categorized under modern natural biodegradable suspending agent, binding agent and film former.

P2641. Determination of mono- and disaccharides in green coffee and their nutritional significance

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Plants produce a wide range of mono-, oligo- and polymeric carbohydrates for a multitude of physiological and structural purposes, with glucose being the key molecule in living systems. Content and composition of carbohydrates contribute significantly to the nutritional value of foods. In addition, flavour and aroma of foods are particularly determined by carbohydrates and its reaction products during processing, e.g. Maillard reaction and glucose degradation products. Profiling free carbohydrates including several provenances of *Coffea arabica* L. and *C. canephora* L. var. *robusta* were performed by high performance anion exchange chromatography coupled to pulsed electrochemical detection.

In the range of free carbohydrates sucrose dominates the low molecular weight carbohydrate fraction of both species, whereas galactose, glucose and fructose are present in very low amounts. This work is part of the EU-Project HEATOX investigating the formation of acrylamide in carbohydrate-rich foods.

P2642. *Rhododendron Kotschy* - Natural Source of Antirheumatic Medicine

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Both *Rhododendron ferrugineum* species growing in western, central and southern Europe and *Rhododendron chrysanthum* species in eastern Asia are frequently used as antirheumatic homeopathic therapy. By using them the side effects of allopathic therapy are avoided.

These two species do not grow in eastern Europe. Instead, *Rhododendron kotschy* species, on which very little pharmacobotanical studies have been done, are present in this area.

Our research includes botanical and pharmacognostical analysis of *Rhododendron kotschy* vegetal product (leaves and flower buds) collected from Romania. We used water, ether and alcohol extractive solutions for qualitative determinations. We also did quantitative determinations and comparative analysis of *Rhododendron ferrugineum* and *Rhododendron kotschy* homeopathic tinctures.

Our finding confirms the similitude of the species taken into study and the possibility of using *Rhododendron kotschy* as antirheumatic natural medicine.

P2643. Inventory of ether essential oil of aromatic fragrant and spice plants of Armenia and their rational usage

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We present to your consideration the results of inventory of ether essential oil, aromatic fragrant and spice plants of Armenia, which by classification of M.M.Ijin are classified by thried groups. We discovered that 190 species with aromatic plants belong to 45 families: Lamiaceae - 36 species, Apiaceae - 34, Brassicaceae - 21, Asteraceae -19, Fabaceae -10, Rosaceae - 9; Ranunculaceae - 8, Polygonaceae - 7, and the rest from 1 to 4 species belong to other different families. There was made up an index for their usage: spice-aromatic, aromatic-fragrant and spices plants, tea usable, food usable /stuffs/, medicinal plants, medicinal-prophylactic plants as well as used in the perfume and cosmetic industries. There is done the index of ether essential oil and aromatic plants by their therapy activity: in the heart and blood system disease; in the diseases kidney, and bladder; in the disease of gynecology and lactamatic diseases, in the violation of metabolism, in the diseases of nervous system and in external usage.

P2644. Production of Rutin by *in vitro* Cultures of *Calotropis procera*.

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In vitro production of phytoconstituents is very important tool to ensure high, reliable and reproducible quality. Rutin is the most promising venoactive antioxidant bioflavonoid. Since production of rutin is very much dependant on its extraction from various plant species including *Calotropis procera* which, contains highest amount of rutin in leaves. In the present investigation therefore, an attempt has been made to raise static, cell suspension and transgenic cultures using *Agrobacterium tumefaciens* and *A. rhizogenes* in MS and modified MS medium from leaf, stem and root explants as an alternative source for better and regular supply of rutin. The estimation of rutin was carried out by HPTLC method, which was developed and validated by the author and the content of rutin was found increased several folds in transgenic cultures. The highest amount of rutin was found in *A. tumefaciens* mediated transformed cultures grown in liquid and semi solid media (MS 1/2) followed by shoots regenerated from leave callus and hairy root cultures. Leaf callus and its cell suspension culture also showed more than 2 and 5 folds increased production of rutin respectively.

P2645. Isolation and determination of fatty acids in oil of seeds and leaves of cotton (var. sahel) and peanut (var. gillane).

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Cotton (*Gossypium hirsutum*) and peanut (*Arachis hypogaea*) are important source of oil currently used in the food industry. In this research oil of seed and leaf of cotton varSahel and peanut var. Gillan were extracted by chloroform and methanol. After measurment, the extract was prepared for analizis and recognition of fatty acid content by GC-MS. This technique were accompanied by realising of fatty acids by saponification and methylation with sulfuric acid-methanol-toluen reagent and then methylated fatty acids were injected to GC-MS instrument. This analysis characteristed that seed oil of cotton var.sahel has a little(<4%) oleic acid and don't have arashidic. The oilseed of peanut has not lignoseric fatty acid.reminder fatty acids presence with higher content than other in this two plants. Unsaturated fatty acids that are essential for growth in mamals, they are arachidic acid, linoleic acid and linolenic acid. Only linoleic acid is actually required in the diet and in this two plants are very high the other two can be synthesised from detary precursors that in this plants was absence.

P2646. Oral administration of *Hippophae rhamnoides* oil extract increases cardiac endogenous antioxidants and prevents isoproterenol-induced myocardial hypertrophy in rats

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Isoproterenol (ISO), a nonselective α -adrenergic agonist, induces myocardic hypertrophy by directly acting on α_1 and α_2 myocardial receptors and by increasing angiotensin II plasma levels. Co-administration of ISO and captopril (an antioxidant) attenuates

cardiac hypertrophy. Our study evaluates the cardioprotective effects of a natural antioxidant extract from *Hippophae rhamnoides* oil (HRO), in isoproterenol-induced cardiac hypertrophy. Male Wistar rats (160 ± 10 g) were treated during 12 days: (1) ISO group- 0.5 mg kg⁻¹ body weight s.c.; (2) HRO group, 50 mg kg⁻¹ orally administered; (3) ISO - HRO group; and (4) Control. Endogenous myocardial antioxidants (superoxide dismutase -SOD, catalase, glutathione reductase -GR, glutathione peroxidase -GPx, peroxi-lipids and SH groups) from isolated hearts were analysed. In ISO group was evidenced a significant depletion of myocardial SOD, GR and GPx activities and an increase of SH levels. ISO-induced changes in myocardial SOD, GPx and GSH were significantly prevented by HRO. In conclusion, HRO treatment protects against ISO-induced myocardial hypertrophy by enhancing antioxidants levels.

P2647. Biological active compounds in cyanobacteria and algae

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During the past 20 years there has been a marked increase in the utilisation of commercial algae products in agriculture, horticulture, pharmacy and medicine, cosmetic industry etc. Recent research has shown that „microalgae“ can produced plant growth regulators. Antibacterial and antifungal activities are also well documented for cyanobacteria and algae.

Effective methods of simultaneous cultivation of different algal strains were modified for the purpose of phytohormones screening. Our present project GA CR 522/03/0323 focuses on the optimization of miniaturized cultures in microtitration plates and assessment of their growth parameters.

Sensitive and specific plant hormone immunoassays were developed in Laboratory of Growth Regulators. Algal suspensions, extracts or medium with their metabolites can be analysed in microtitration plates coated with individual anti-PGR antibodies. ELISAs for zeatin ribside, isopentenyladenosine, benzyladenosine, topolins, indole-3-acetic acid and abscis acid are available. Overproducing strains selected by these methods can be subsequently subjected to detailed analyses by HPLC/ELISA or HPLC/MS.

P2648. Standardization and Immunomodulatory activity of *Mangifera indica* Linn. cultivar Neelam

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Studies have indicated that some of the strongest immune system boosters come from specific healing herbs. A number of these plants are known as adaptogens, which strengthen and normalize the nervous and hormonal systems thereby helping us to adapt to (withstand) the many diverse stresses of modern times. Some also contain antioxidant components which can slow down the cellular aging process and bolster the immune system as well.

Mangifera indica Linn. (Anacardiaceae) is a large evergreen tree of tropical and sub-tropical regions. Ripe mango fruit is considered invigorating, refreshing and fattening. The juice is recommended as a restorative tonic. It is useful in apoplexy.

In the present study, the alcoholic extract of fruit of *Mangifera indica* Linn. has been investigated for its effect on cell mediated and humoral components of the immune system in mice. Administration of test drug produced increase in humoral antibody (HA) titre and delayed type hypersensitivity (DTH) in mice. *Mangifera indica* was found out to be a good herbal Immunomodulator and Adaptogen with immunostimulating properties.

P2649. Gathered food plants in Eastern Spain wild, weeds and crypto-crops

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The “Ensalá del Campo”, “Pastissos d’herbes” and “Camarrotjes” are collective names that in Spanish and Valencian serve to design gathered food plants in the eastern coast of the Iberian Peninsula. These are over one hundred species, with the higher

proportion of Compositae, Cruciferae and Liliaceae. Their composition and pharmacological activity have been investigated in the project Local Food - Nutraceuticals.

Although these plants configure a wide repertory of local food recipes, most of the species are widespread in the Mediterranean and examples of similar uses are also recorded in central and eastern Mediterranean countries. The endemic herbs used to give particular flavor to the dishes, such as *Thymus piperella* L. or *T. moroderi* Pau ex Martínez contain relevant active substances.

Ethnobotanical questionnaires have been administered to over two hundred persons in thirty different localities of this area in order to obtain a detailed information on gathered food plant preferences and traditional processing. Over one thousand questionnaires were recovered and introduced in a data base that allows to analyze their contribution to the diet.

P2650. Evaluation of hypericin content (from *Hypericum perforatum*) from mice plasma

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Hypericum perforatum extract is used as an antidepressant and a nutritional adjuvant. **Method:** *H. perforatum* extract (Bionorica, Germany) is standardized at 0.4% hypericin content. A HPLC method was used for the detection of hypericin levels from *H. perforatum* extract and from mice plasma. Hypericin was separated by C18 chromatography column (25 cm x 4 mm) using a new mobile phase, ammonium acetate / acetonitrile (4:6; pH = 5). The quantification was performed by using hypericin methanolic external standards (Planta Natural Products, Austria). The method allows the detection of hypericin fluorescence (ex = 315 nm; em = 590 nm). **Animals:** *H. perforatum* extract was orally administered during one month to 28 mice (1 month and 3 month aged) males and females. **Purpose:** The goals of our study was to identify the age and sex differences of hypericin plasma levels. **Results:** This is the first study on mice in order to determine hypericin plasma levels. Retention time on HPLC column of hypericin is evaluated at 10.85 min for the standard and 12.44 min in plasma mice. Age and sex differences of hypericin plasma levels were observed.

P2651. Nutritional Value of *Asparagus acutifolius* L.

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About forty plant species are used as vegetable in Turkey. In the Mediterranean and regions *Asparagus acutifolius* L. which is one of the perennial plants and belongs to Liliaceae family, is used traditionally as vegetable. And this plant is also known as wild asparagus.

There is no study on the nutritional value of this vegetable which is one of the newest vegetable culture of *Asparagus acutifolius* L. The aim of the present study is to determine nutritional values of different organs's (rhizomes and green asparagus spears) of *Asparagus acutifolius* L.L.

The results of this studies, some botanic characteristics (plant height, plant weight, root length and flower size) and nutritional value (N, P, K, Ca, Fe, Na, Mn, Mg, Zn, S and total soluble solid content) were determined in *Asparagus acutifolius* L. We were also determined carbohydrate content and titratable acidity and total ash.

P2652. Traitmill(tm): A Functional Genomic Platform For The Phenotypic Analysis Of Gene Function In Plants.

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The modification of quantitative traits in crops through biotechnology is today highly challenging. To meet this challenge, CropDesign has developed TraitMill, an integrated platform that allows high-throughput and high-resolution testing of the effect of plant-based transgenes on agronomical traits in crop plants. Traitmill currently focuses on rice, which is a good model for other important cereals such as corn and wheat. It involves the following components:

i) A bioinformatics-aided selection of candidate trait improvement,

- ii) A collection of promoters validated in rice,
- iii) A high-throughput production of transformation vectors,
- iv) An industrialized plant transformation system that generates tens of thousands of transgenic plants per year
- v) An automated plant handling, imaging and analysis set-up for trait evaluation

Transgenic rice lines showing increased seed yield or increased green biomass have been obtained from TraitMill. Field experiments showed that the plant phenotype observed in Traitmill was often conserved in the field. Thus TraitMill can be used as a good proxy for the validation of transgenes useful for crop improvement.

P2653. Investigation on the potential utilization of Paulownia wood in Particleboard production

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A number of factors were responsible for utilization of material in particleboard industry. Species, press time, resin type, hardener, press temperature, resin content and other factors are important. In this investigation feasibility of Paulownia fortunei wood in particleboards production were prepared at four different Combination of 100%, 75%, 50%, and 25% (base of paulownia), three different press time of 4.5, and 6 minutes.

Based on the DIN-68763 standard physical and mechanical properties including Modulus of rupture (MOR), Modulus of elasticity (MOE), and internal bonding (IB), and thickness swelling (after 2 and 24 immersion in water) were tested.

Based on the factorial experimental design at completely random test was analysis and DMRT test was applied.

The results showed that potential utilization of paulownia wood in particleboard production was optimized. For particleboard industry utilization of paulownia is possible.

P2654. Investigation on medium density fiberboard properties produced from eucalyptus microtheca wood

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In this investigation, Eucalyptus Microtheca wood for medium density fiberboard (MDF) production and determining of anatomical characteristics, mechanical and physical properties of boards have been studied. variables factors were steaming time in two levels (7.5 and 15 minutes), press temperatures in two levels (165 and 175 °C) and press time in three levels (3, 4 and 5 minutes).

The results of this study indicated that, the highest bending strength (MOR), were observed on MDF boards produced for 7.5 minutes steaming time, and the lowest of MOR belong to boards produced for 15 minutes steaming time. However the maximum and minimum modulus of elasticity (MOE) achieved with fibers obtained under same condition. In all conditions, increasing press time was to improve MOR and MOE of the boards.

The results also showed that the maximum internal bonding (IB), were related to MDF boards produced with fibers obtain under 15 minutes steaming time condition. However increasing of press time did not affect on IB significantly. Thickness swelling (T.S) after 2 and 24 hours soaking in water of boards had inverse relationship with IB.

P2655. Varietal differences in bast fiber production in kenaf (*Hibiscus cannabinus* L.)

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Kenaf (*Hibiscus cannabinus* L., Malvaceae) is an important fiber crop that originated in southern Africa and is currently widely cultivated in tropical regions. The stalk contains two different fiber types, an outer "bast" and an inner "core". The fibers are either used separately or together in the manufacture of different products ranging from paper to woven fabrics and industrial absorbents. The cultivation of kenaf is primarily concerned with the bast fiber yields because they are used to produce high-grade fiber. In this study, growth and bast fiber yield of several kenaf

varieties was investigated. Nine kenaf varieties were sown on May 19, June 19, and July 19, 2000. Growth was investigated by estimating dry matter production and bast fiber yield under different temperature regimes. Growth was observed to differ markedly among varieties and the percentage of bast fiber in dry matter ranged from 31.4 to 40.4%. The different trends in bast fiber content observed among varieties were independent of time of sowing.

P2656. Antioxidant Activities of *Leonotis nepetifolia* leaf

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Leonotis nepetifolia (Linn.) Ait. f (Labiatae) leaf is used in Nigeria to cicatrize wounds and sores (1). The correlation between oxygen free radicals and damage to skin is well documented. The leaf was extracted with 80% ethanol. The anti-oxidative activities, including the radical-scavenging effects, reducing power and Fe²⁺-chelating ability were studied in vitro. It was found that the anti-oxidative effect of the leaf extract of *L. nepetifolia* was strongly concentration dependent. In general, the anti-oxidative activity increased with increasing extract concentration. From a comparison of the IC₅₀ values for each anti-oxidative reactions, it appeared that the leaf extract was more effective in reducing radicals than chelating Fe²⁺ or scavenging -diphenyl-picrylhydrazyl (DPPH) radicals. However, compared to the commercial antioxidant, the extract showed less scavenging effect on the DPPH radical and reducing power but better Fe²⁺-chelating ability than -tocopherol.

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P2657. Histological characterization of Guayacan wood sample obtained from four different markets in México City.

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Mexico is one of the countries where medicinal plants are sold as an alternative to attend health problems. However, it's always a confusing task to identify the species we're dealing with in the market place. *Guaiacum coulteri* wood and cortex (so called Guayacán) is used for the treatment of type 2 diabetes. The present study focused on characterizing anatomically samples of Guayacán obtained from different markets in Mexico city, as well as comparing these samples with some obtained from the Biology Institute xylarium, UNAM. The wood samples obtained from markets were processed and cut in a microtome for further analysis in the light microscope. The anatomical structure from samples obtained in the market was different from *G. coulteri* samples from the xylarium in respect to porosity clusters, parenchyma bands and heterocellular ratios meanwhile *G. coulteri* shows no clustered porosity, unilateral wingshaped parenchyma and homocellular ratios. Results show that market samples are anatomically different from *G. coulteri* samples. Attention must be paid in respect to quality control in medicinal plants sold in local markets.

P2658. dna fingerprinting of some vigna germplasm by rapd and issr.

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Phenotypic and molecular evaluation of *Vigna radiata* and *V. mungo* will help identify accessions potential relevance for *Vigna* improvement programme. 100 germplasm of *Vigna radiata* and *V. mungo* of high yielding, local collections and wild ancestors comprised the experimental materials for DNA fingerprinting using RAPD and ISSR primers. RAPD reactions were done following standard procedure with annealing temperature of 36°C. Primers comprising of mainly (GA)₃T, (GATA)₄, (AG)₃C, (TTA)₄TT, (AG)₃T were used for ISSR reaction adopting annealing temperature according to the T_m of the primers. Extensive inter and moderate intra group variation were detected which may help in cultivar identification, monitoring and aiding gene introgression between germplasm of pulses. Dendrogram as revealed by ISSR data correlates better with phenotypic clustering than RAPD data. ISSR markers gave higher polymorphic bands than RAPD markers. PIC

as revealed by ISSR primers is greater than that of RAPD primers. *V. mungo* germplasm has narrower genetic base than *V. radiata* germplasm. One decamer RAPD primer gave very high PIC values (0.39) when compared among greengram germplasm.

P2660. Economic Potential Plants in Conservation Areas of the Brazilian Cerrado

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This work studied plant species with economic potential. and was carried out in Conservation Areas of the Brazilian Cerrado. The methodology included field collections, bibliographic survey, herbarium survey, morphological analysis and identification of the species. It was found species of following families: Anacardiaceae (*Anacardium humile* St. Hil.), Annonaceae (*Annona coriacea* Mart.), Apocynaceae (*Aspidosperma tomentosum* Mart.), Asteraceae (*Achyrocline satureioides* (Lam.) DC., Bignoniaceae (*Tabebuia rosea* A. DC.), Dilleniaceae (*Davilla elliptica* St. Hil.), Leguminosae (*Dimorphandra* spp.), Moraceae (*Brosimum gaudichaudii* Trec.) Solanaceae (*Solanum lycocarpum* St. Hil) and Vochysiaceae (*Vochysia rufa* Mart.). Descriptions, figures, synonyms, common names, plant parts used by people and data on species distribution are showed. Species distribution maps in conservation areas was elaborated. Population structure data for *Dimorphandra mollis* Benth, *D. Gardneriana* Tulasne, *Caryocar brasiliense* Camb, *C. coriaceum* Wittm. also are available, which could be applied for species management by small farm communities.

P2661. Biochemical changes in cocoa seed tissue in the course of fermentation

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Fresh harvested cocoa seeds are transferred into raw cocoa beans by a plant biotechnological fermentation process. In the course of this fermentation the seed adhering pulp is degraded by microbial action. Within this process lactic acid and acetic acid following ethanol oxidation are produced. Acetic acid penetrates the cocoa seeds and induces proteolysis and aroma precursor formation. The fermentation results in heterogenous raw cocoa beans, which are by visual means classified into slaty, violet, and brown stages. These stages are characterized by considerable changes in biochemical features. The free amino acid content increases with special respect to Ala, Leu, Ileu, the free extractable phenolics are strongly lowered and the enzyme activities, especially the seed phenolase is brought to minimum values. In the transition from slaty to brown seeds the violet stage allows to analyse the way of the post harvest treatment. This means by the complex biochemical features of the violet seed stage in raw cocoa a "processing history" of the traded material can be postulated.

P2662. Photosynthetic light response curves of different pot plants

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Plants grown in interior spaces actually come from tropical or arid regions and must adapt to less than ideal conditions in the home or office. Light-response curves give us a wealth of useful information about the photosynthetic mechanisms operating inside the leaves. The net photosynthetic rate (Pn) of intact leaves of experimental pot plants grown in greenhouse was measured by infrared gas analyser in photosynthetic photon flux density (PPFD) from 0 to 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and constant other main conditions. Maximum Pn of *Tradescantia hybrida*, *Primula obconica* and *Radermachera sinica* at 1200 PPFD varied from 5,23 to 8,33 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas *Campanula carpatica*, *Dendranthema indicum* and *Rosa hybrida* reached higher saturation photosynthetic rate from 14,6 to 22,79 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Determination of light response curves is important for selection of optimum light conditions for interior plant cultivation and maintenance.

P2663. Fruit maturity and interrelated of temperature and container on longevity of NEEM (*Azadirachta indica*) seeds

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Neem (*Azadirachta indica* A.juss) native to the arid region of Indian subcontinent is a valuable tree because its fast growing rate and many technological importance application. Fruits of neem were collected at different intervals of maturity. Physiologically mature seeds with maximum germination capacity and Longevity were obtained 10 to 12 weeks after flowering (late July to early August) when

some of the fruits turned yellow and started falling on the ground . This period coincided with peak green weight , size and a fully grown embryo enclosed in hard fibrous endocarp . Seeds were stored at 27°C , 17°C and 2°C in sealed polythene bag perforated polythene bags perforated cardboard boxes (seeds wrapped in blottis). It was found that sealed containers and low temperature 2°C are responsible for rapid deterioration of seeds while well aerated containers inhibited deterioration up to certain extent at room 27°C and 17°C till 6 months.

P2664. Quantitative and qualitative study of peroxidase and polyphenols in Iranian tea (*Camellia sinensis*) during various stages of growth

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Tea is consumed worldwide and ranks only second to water as a beverage. It is prepared from the dried leaves of *Camellia sinensis*. Tea peroxidase and polyphenols are strong antioxidants and are believed to have beneficial health effects. In this research, leaves of tea were collected three times during growth season and for preparation of crude extract the leaves were homogenized in 0.02 M Tris-HCl buffer (pH 6.8). Quantitative and qualitative alteration of peroxidase during growth stages was determined by spectrophotometry and PAGE system, respectively. The effect of pH, temperature and some chemicals (NaCl, Na₂S₂O₃, benzyl adenine and chloride of bivalent cations) on peroxidase activity using H₂O₂ as substrate were investigated. The content of polyphenols as the non-enzymic antioxidants was also determined in leaves during several plucking. Peroxidase activity and the content of polyphenols in the first and third harvest were higher than that of the second harvest.

P2665. Physiological responses of sweetpotato *Ipomoea batatas* Lam. under different kinds of irrigation

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Sweetpotato (*Ipomoea batatas* Lam.) can be grown in poor soils with little water. This work was held from February to July in 1995. Objective was to determine physiological responses of sweetpotato when it is subjected to different kinds of irrigation. Cuttings of 17 genotypes were planting in dry sandy soil and irrigated immediately after planting. Treatments were: irrigation at 45 d.a.p., irrigation at 90 d.a.p., without irrigation for all experiment and irrigation every 15 days. A reduction in plant coverage in treatment without irrigation and excessive growth of foliage in treatment irrigated each 15 days was observed in all genotypes, hence water limit growth of foliage. Some genotypes shown high stomata resistance in treatment of irrigation every 15 days, this is because some genotypes are sensible to high soil water content. Leaf water potential is no signified between treatments, inclusive genotypes with high stomata resistance. Dry matter content in roots is no signified between treatments, it means that photosynthesis is not totally affected, but possibly accumulation of water in tissues could be affected.

P2666. Larvicidal and repellancy effect of essential oil of *Cymbopogon winterianus* against mosquitoes and isolation of bioactive compounds

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Cymbopogon winterianus is being extensively used as traditional phytopesticide in the North Eastern part of India and many other parts of the globe. Its essential oil was extracted and its bioefficacy against *Aedes aegypti* and *Culex quinquefasciatus* was studied

with the standard procedure recommended by WHO. The calculated LC₅₀ was 46.88ppm against *A.aegypti* and 13.27 ppm against *C. quinquefasciatus*. It showed 80% & 30% repellent activity over 3hrs & 4hrs against *C.quinquefasciatus* and 70% & 25% activity over 3hrs & 4hrs against *A.aegypti*. GCMS profile identified 59 compounds (citronellal 26.51%, citronellol 11.38% and geraniol 2.27%).

Of the six fractions (F I-VI) of the oil after fractional distillation, FV exhibited LC₅₀ of 12ppm followed by FII with LC₅₀ of 13.4 ppm against *A.aegypti*. Against *C.quinquefasciatus* FII showed the lowest LC₅₀ (10.10ppm) followed by that of FV (14.03ppm). FI exhibited potency with 60% repellent activity over 4 hrs against *A.aegypti* and 55% against *C. quinquefasciatus*.

The fractions after being subjected to GC analysis were identified as geraniol (FV), limonene(FII) and citronellal(FI) by taking their standard retention time.

P2667. *Rhipidocladum racemiflorum* (Poaceae: Bambusoideae) a natural resource of Mexico with regional economic importance

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Although different aspects of Asian bamboos have been studied, mexican bamboos are relatively unknown. Information on the uses of Mexican bamboos is scarce. Bamboo uses were detected in the Monte Blanco locality of Veracruz state. The objective was to document uses of mexican bamboos in Monte Blanco and its economic importance. Methods used were interviews, exploration of natural areas where bamboos grow, identification of bamboo species, etc. Results showed that bamboo use is to elaborate furniture and handicrafts to substitute coffee plantations or in some cases as a complementary activity to increase the family budget. Six bamboo species are used but only one species, *Rhipidocladum racemiflorum*, is native to Mexico. They call it "chiquian" for its thin culm and flexibility. It is used to elaborate chairs as well as delicate decorations for most furniture, handicrafts like lamps, flowerpots, screens, kitchen cupboards etc. It is a cheaper material because it is gathered from its natural habitat. Conclusions are propagate *R. racemiflorum* in Monte Blanco in order to increase income and protect the species habitat, as well as to promote the Monte Blanco furniture.

P2668. Bioactive molecules in *Passiflora* ssp.

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The genus *Passiflora*, with several hundred species many of which of ethnobotanical importance, looks very promising for the identification of bioactive molecules. To this purpose, we are working on: a) development of efficient *in vitro* or *in vivo* propagation methods; b) screening of antioxidant, antimicrobial and antitumor activity in methanol extracts; c) bio-guided identification of the active molecules.

From seeds of about 60 species, mainly collected in the wild, we obtained stable calli for 28 species; 13 of these also regenerated plants. Leaf methanolic extracts of *P. mayarum*, *P. foetida*, *P. nitida*, *P. platyloba* showed inhibitory activity on *E. coli* growth. *P. nitida*, *P. garckeii*, *P. palmeri* and *P. subpeltata* showed high antioxidant activity in a microplate DPPH assay. *P. nitida*, *P. foetida* and *P. palmeri* showed also apoptotic effect on MDA-MB231 tumour cells. The extracts of stems or leaves of other species showed anti-STAT1 (*P. tenuifolia* and *P. palmeri*), anti-STAT3 (*P. palmeri* and *P. nitida*) and anti-STAT6 (*P. palmeri* and *P. coriacea*) activity. Identification of the bioactive molecules is in progress and their production in calli is being explored.

P2669. Acquaintance with biotechnological abilities of *Dunaliella* alga

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In order to acquaintance with biotechnological abilities of *Dunaliella* alga for beta-carotene product in Iran, at the first four species of *D. viridis*, *D. parva*, *D. pseudosalina*, and *D. salina* isolation, purification and exactly identified from salt march of Gave Khoni and then physiological effects of salinity on cells growth, glycerol and beta-carotene contents studied in the purified algae

distinguished that species of *D. parva*, *D. pseudosalina*, *D. salina* are able to beta-carotene product and the species of *D. salina* has the most product amount of beta-carotene. In view of the fact that, Iran has abundant lakes and salt marshes and it has suitable climate for growth of *Dunaliella* algae and also, it has sun intense light radiation for beta-carotene production, so it seem that the use of *Dunaliella* species especially *D. salina*, is possible to culture widely in a numbers of little pool in order to commercial production of beta-carotene.

P2670. Toxicity of *Petiveria alliacea* L. on white fly (*Trialeurodes vaporariorum* West.) in laboratory and greenhouse

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Preparations from *Petiveria alliacea* have been used extensively in traditional medicine and the insecticidal and acaricidal properties have been reported. To determine the toxic effect of *P. alliacea* on *Trialeurodes vaporariorum* the mortality rate and the CL₅₀ were evaluated in the laboratory and under greenhouse conditions by preparing three extracts: aqueous, methanolic and dichloromethane. For the laboratory, 50 aleroididos were placed in petri dishes with a foliole of tomato, that was previously sprayed with solutions. In the laboratory, the extracts mortality rates were higher than under greenhouse conditions. The CL₅₀ was higher under greenhouse than laboratory conditions. The CL₅₀ for aqueous, methanolic and dichlorometane were 4.6 %, 1.1 % y 0.3 %, respectively, in laboratory, and 16.6 %, 13.3 % y 3.5 %, respectively, under greenhouse conditions. The organic extracts were more toxic than the aqueous extracts in both bioassays against *Trialeurodes vaporariorum* adults. The treatments didn't interfere with the tomato yield (grams and diameter per fruit), inferring the feasibility of using extracts against *T. vaporariorum* control.

P2671. High levels of variability observed in plants obtained by asexual reproduction of *Agave tequilana* var. azul

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Agave tequilana Weber, is an important economic crop in Mexico, since it is used to produce tequila. *Agave tequilana* is a diploid (2n=60) with a long life cycle of 8 years. Plants flower only once towards the end of their life and produce seeds with low viability. *Agave tequilana* has been propagated asexually through the production of offsets from rhizomes for more than a century with very little sexual reproduction occurring. Another mechanism of asexual reproduction occurs in the inflorescence producing bulbils, but this method is not used for propagation in commercial plantations. It would be expected that offsets and bulbils of the same plant are genetically homogenous, however AFLP analysis shows differences in AFLP patterns between individuals obtained by both types of asexual reproduction. The genetic variability generated in bulbils is higher than in offsets.

These findings suggest that it may be possible to select individuals with desirable characteristics from asexual populations and raises the question as to how these high levels of variability are produced.

P2672. Genetic diversity on Tunisian pomegranate (*Punica granatum* L.)

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To study the diversity of pomegranate cultivars, we used phenotypic features of seeds as well as RAPD markers to studying the molecular diversity.

Morphometric analysis of seeds is based on analysis of 7 seed characters followed by statistic analysis. Multivariate analysis are carried out. PCA (Principal Compound Analysis) show distinction of some cultivars. It is the case of cultivar Gabsi 1 (GB1) for example. Group analysis and drawing up dendrogram shows several cultivars clustering.

Besides a PCR-RAPD approach was optimised and used to estimate the genetic relationships among 12 tunisian pomegranate cultivars. Four random primers generated 29 RAPD loci among

them 24 are polymorphic. The resolving power of the four primers ranged between 1.333 and 4.832. A similarity matrix was constructed on the basis of the presence or absence of bands. Among pomegranate cultivars the mean of genetic similarity is 0.516. The polymorphisms in PCR amplification products were subjected to the unweighted pair group method for arithmetic average (UPGMA) and plotted in a phenogram. Cluster analysis identified two main clusters.

P2673. Using pattern, depletion and conservation strategy of the *Triphala* in the village groves of Chittagong region, Bangladesh

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Triphala is an important combination of three important fruits, i.e., *Emblica officinalis*, *Terminalia chebula* and *Terminalia bellirica*, which is extensively used as medicine in Bangladesh. Chittagong region in Bangladesh was once rich in *triphala* trees presently subject to the over extraction, lack of proper management, and unawareness. Thus these forest resources are being threatened day by day. The study was conducted to ascertain the present using pattern of the *triphalas*, causes of its depletion and the conservation strategies agreed by the village dwellers. A total of eight major uses of *triphala* trees were recognized. It was revealed that 100% respondents used the *triphala* as fruit tree followed by 75-79% as fuelwood. The present status of growing stock of *triphala* was found in depleted condition particularly in the Muslim dominated area. Induction of social forestry program, proper maintenance and supply of planting materials were found major recommendations by the villagers to retard the depletion of the *triphala* species in the Chittagong region, Bangladesh.

P2674. Study and valuation of Amaranth cultivation in Iran

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The aim of the project was to investigate the possibilities and problems of the production of grain-amaranth in Iran (Pakdasht Province of Varamin area) with respect to plant cultivation.

In trials plant densities of 200-300 thousand proved to be favorable, higher densities improved drying of the grain, but can be a shortcoming under drought conditions. Harvesting should be done as late as possible: the longer the growing period from seeding until harvest (approx. end of July) the higher can be the yield. At a growing period of 127-147 days yields averaged 2000 kg/ha. Under optimum conditions, a maximum yield of 4000 kg/ha could be obtained on a fertile soil. Excessive use of fertilizers delay flowering and field grain drying. Pre-conditions for optimized harvesting in a dry-climate-region - genotypes with fast and homogeneous field grain drying - could be identified.

Various cultivars of the species *Amaranthus cruentus* and *Amaranthus hypochondriacus* were tested on about 6 characteristics. A bad drought resistance of *Amaranthus hypochondriacus*, with an average flowering delay of 15 days, was found. *A. cruentus* was found to be more adaptable for Iranian agriculture.

P2675. Jojoba: A new crop for Mediterranean Region of Turkey to conserve soil and income for growers

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Jojoba (*Simmondsia chinensis* L.) is a dwarf, plain looking, evergreen perennial shrub. The Taurus mountains lay down throughout the Mediterranean Coastal of Turkey, and soil erosion takes place at this basins. Since some areas have water shortage and bad soil structure these plants can not easily grow in that areas, but Jojoba is suitable plants for these lands.

Jojoba seeds were sowed in Kumluca-Antalya in 1990. The germinated seeds were transferred to the plastic tubes in 1991, and when their lengths were reached to about 40-50 cm they have planted to the fields in early March in 1991. The plants were fruited in 1997 and 3 female types were found suitable for the region in

terms of yield, oil content, resistance to diseases, pests and low temperatures, fruiting uniformity and multi-fruit settings in buds. This promising types were propagated by cuttings.

P2676. Biotechnological strategies for improvement of Annatto colour production

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Bixa orellana Linn (Fam. Bixaceae) is a small tree native to tropical America commonly called as annatto or achiote. Seeds of which give reddish orange color that are commonly used in vegetable oils, drinks, dairy industry, confectionaries and cosmetics. It is the only source of the apocarotenoid bixin (ca.82% of the annatto pigment contains bixin) - a principal coloring constituent of annatto - being produced at the aril portions of the seed. Annatto ranks second in economic importance (next to saffron). Since it is a cross-pollinated one, there is a need to standardize efficient protocols for mass multiplication of elite trees with high yield, enhanced pigment production and disease resistance. Improved silvicultural practices will also yield good returns. The enzyme lycopene cleavage dioxygenase is said to play a key role in the bixin biosynthesis pathway from the precursor lycopene - the richest sink of tomato. Many things are yet to be played upon in this pathway to reveal the nature's secret using the tools of today's biotechnology. The significance of lycopene cleavage dioxygenase will be discussed with reference to *Bixa orellana*.

P2677. Biotechnological intervention for cloning and germplasm preservation of *Pterocarpus marsupium* Roxb.

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Pterocarpus marsupium Roxb. (Fabaceae), is a multipurpose forest tree of immense medicinal and excellent timber value. The highly heterozygous nature and lack of conventional methods of vegetative propagation warrant application of biotechnological means for cloning and germplasm preservation of elite tree of proven qualities. In this context, a protocol for cloning via multiple shoot formation employing cotyledonary node (CN) explants taken from aseptic seedling was developed. CN were induced to regenerate offshoots in a well known nutrient formulation augmented with various cytokinins viz. TDZ, kinetin and BA. Superiority of TDZ over other cytokinins in inducing multiple shoot buds and their differentiation was observed. Regenerated shoots were proliferated under the influence of BA and IAA. The *in vitro* proliferated and elongated shoots > 4 cm, planted individually on root induction medium. The shoots were rooted nearly to 50-60 percent success to form complete plantlets by adapting two step culture procedure. The root-regenerated shoots showed sustained proliferation and complete plantlets were raised and grown *ex-vitro*.

P2678. Anthraquinone formation in *Senna alata* tissue cultures.

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A high performance liquid chromatography was established for the simultaneous quantitative determination of the anthraquinones, including aloe-emodin, rhein, emodin and chrysophanol, produced in *Senna alata*. The root cultures of *Senna alata* were established from the high anthraquinone-yielding *S. alata* roots initiated in liquid B5 medium supplemented with 0.5 mg/l NAA and 1 mg/l kinetin. The obtained root cultures produced higher amount of emodin (0.27 %w/w) and chrysophanol (0.68 %w/w) than that in the leaves and root of the intact plants. Unfortunately, the root cultures have dedifferentiated to be cell cultures after several subcultures with a reduction of emodin and chrysophanol production. Improvement of the medium for an increase of anthraquinone production in the cell cultures exhibited that B5 supplemented with 0.5 mg/l kinetin was suitable for emodin and chrysophanol production. In addition, a declination of emodin and chrysophanol production in the cell cultures was still observed in succeeding subculture.

P2679. Quality evaluation of herbal drugs- A present day need

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Commercial production of medicines and other products based on different systems of medicine not only increased many folds in India but world over. Increase in urbanization and population has also increased the tendency of profiteering. A large number of manufacturing units, have entered in the area of herbal drugs and pharmaceuticals. A majority of raw materials employed by these industries are of plant origin, the bulk of which comes from wild sources. The supplies are usually obtained through various trade channels. This has created serious problems with regard to the quality of the raw materials and their desired therapeutic effects. In addition to these, nomenclature of drugs is one of the common factors for creating lot of confusion to the botanical identity of the herbal raw drugs and thus further aggravating the problems of adulteration/substitution in commerce.

Not only morphology can play important role in authentication of herbal drugs but some other parameters like HPTLC; GLC, etc. can also be utilized as marker parameter for drug standardization.

P2680. The effects of hydro alcoholic extract of soya in the histological structure and the level of estradiol and testosterone in male sorie

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Soja is a rich source of phytoestrogen. Therefore, the objective of this project was to determine whether soja extract can change the structure of male reproductive tissue and it can influence on the level of sex hormone. For this purpose, 50 male sories were selected and divided into 4 groups. 3 experimental groups were fed with 150, 100 and 75 mg/kg of hydro alcoholic extraction of soja and control group were fed with the water for 50 days. Blood samples were taken in zero and fiftieth day of experiment and the level of sex hormone were measured and reproductive tissue were removed, fixed, processed and stained with H and E staining method. Histological of sections were revealed some change in testis. In the testis many spermatocytes at the metaphase stage in 150 mg/kg and 100 mg/kg treated groups and some of these mitotic figures. In conclusion, it seems that soja extract can change the spermatocyte, in low concentration, it may induce proliferation and high concentration, it may lead to cell death and nucleus fragmentation. The result indicated that level of testosterone and estradiol was decreased significantly in the group that was fed with 150 mg/kg.

P2681. Morphological Diversity in cultivated and wild species of Sesamum

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Sesame (*Sesamum indicum* L. syn *Sesamum orientale* L.) is one of the oldest oilseed crops and is under cultivation in Asia from ancient times. Oil and protein are important attributes. The genus *Sesamum* comprises 36 species and only limited studies are available on the desirable traits present in different species. Wide diversity is available in the existing germplasm. Our studies in five cultivars (*S. indicum*) viz., CO 1, SVPR 1, TMV 3, TMV 4 and TMV 6 and four wild species viz., *S. alatum*, *S. malabaricum*, *S. occidentale* and *S. laciniatum* on growth habits, height, floral morphology, capsule size, colour and seed anatomy indicated the presence of wide variability which can be used for developing high yielding varieties with resistance to phyllody disease. Experimental results and future breeding strategies for sesame will be presented.

P2682. Regeneration potential of Calotropis procera: an important bio-fuel yielding plant of semi-arid regions.

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Calotropis procera grows in most arid conditions under temperatures ranging from 45 to 50 degree Celsius. It survives by underground thick roots which provide new branches once the aerial portions are completely dried up in the winter season. The *Calotropis* has great potential to produce biomass under adverse

climatic conditions. The yield of the plant can vary from 2.7 dry tonnes to 35 dry tonnes per ha per annum in 3 year rotation cycle. Considering the high temperatures the *Calotropis* is an ideal plant for the desert regions. It yields several medically important products also. The biofuel production by this plant has been studied in detail. The time of cutting, length of cutting and the level of cutting under field conditions were evaluated under local region in Vatika and the regeneration potential was studied. The season for harvesting greatly influenced the regeneration potential which was substantially low under winter conditions as compared to spring conditions. The yield of the plant could be doubled by making proper harvesting schedule. Details shall be presented.

P2683. Resource assessment of bio-diesel yielding species Pongamia pinnata and Madhuca indica - a critical factor in large scale cultivation

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Pongamia pinnata and *Madhuca indica* are promising indigenous bio-diesel yielding species from India. They could play an important role in meeting ever growing energy requirements of India in a sustainable manner if exhaustive resource assessment of these species is carried out.

Commercial cultivation of these plants specifically for this use is yet to begin in India. Govt of India proposes to cultivate *biodiesel* species on 13 million ha of degraded wastelands of the country. Over 200 districts in 19 States of the country have been identified on the basis of wastelands availability, rural poverty ratio and agro-climatic conditions for taking up such cultivation.

It is important to have realistic yield estimates and economics of production through a series of multi-location yield trials under different agro-climatic conditions, before launching national programme. Present communication has provided the details of such assessment needs and methodologies to be used for such assessment outside the forest or protected areas.

The present paper also deals with the methodology to know the exact availability of the species in wild as well as in community circumstances.

P2684. Eradication of Basidiomycetes and fungus in fruit trees flowers and vegetables.

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Biotens is a biological surfactant and 3TAC is a biological fungicide, made by Avance Biotechnologies, Chile. Attack of *Chondroterium purpurium* (lead disease) on fruit trees appears as silver on its leaves; as fungus obstructs vascular bundles releasing toxins trees die. Due to its vascular position there was no treatment to eradicate the disease. Use of 3 TAC (3 applications to foliage) and Biotens as surfactant allows translocation in wood and elimination of fungus. Result: samples for electronic microscopy pre and post treatment show that regression of disease is related to regression of silver characteristic signs and symptoms, furthermore the fruitness attached to trunk fall after second application new sprouts are green and at cut they do not present the characteristic ring of disease. In case of fusarium in tomatoes and carnations in a vascular position, repeats results on trees.

P2685. Micropropagation system of Curcuma alismatifolia Gagnep. by using Temporary immersion bioreactor

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This particular study involved the duration and feeding frequency of twin-flasks temporary immersion bioreactor in order to improve the micropropagation system of *Curcuma alismatifolia* Gagnep. Retarded shoots of *Curcuma* were cultured on a modified MS (1962) medium supplementary with 0.5 mg/L thidiazuron and 4 mg/L Imazalil. Treatments consisted of 4 different sets of duration and feeding frequency such as 2 1-minute feeding/day; 6 1-minute/day; 2 15-minute/day; and 6 15-minute/day. These treatments were compared with those cultured on solid and liquid media for a 6-week period. The experiments were repeated 2 times. Results showed that 6-time feeding/day for either 1 or 15 minutes gave high number ranging from 1,126.3 to 1,518.3 shoots per container. In the contrary, shoots cultured on the solid medium produced only 241.8 shoots per container with a slight increase in the shoot number being observed in liquid culture. It was also

noticed that frequency of feeding provided more effect to the quality of the plant than feeding duration.

P2686. Tropicos: Next Generation - A global resource for taxonomic reference data

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Since 1982 **Tropicos** has been Missouri Botanical Garden's primary data repository for taxonomic information, now including nearly a million names with synonymy, types, references and 2.4 million linked specimens. Web-based **W3Tropicos** debuted in 1997 and now sees over 40,000 queries daily from taxonomists worldwide. **Tropicos: Next Generation (TNG)**, the newest version of MBG's widely used system, implements a feature-rich GUI, web services, a robust, responsive relational data model, and an n-tier system component model. It has been designed to support interfaces both to and from other data sources/systems via web services and out-links using TDWG/GBIF standards. Employing a Services-Oriented Architecture, **TNG** enables load balancing and rapid extension of both processing and network responsiveness. Plans include full data integration from taxon records to on-line flora and identification key systems, digital images of type and other specimens plus digitized literature and other botanical references. Support for direct Internet-based editing by specialists is also planned to leverage the knowledge of the world-wide systematic botany community.

P2687. The systematization and construction of metadata of the Herbarium of the Pontificia Universidad Javeriana - HPUJ

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Herbarium Pontificia Universidad Javeriana, Bogotá, Colombia.

The systematization and construction of metadata of biological collections improve precious information and gives virtual access to global resources. A database allows analyzing the information to get estimations about taxonomical, ecological, ethnological and geographical aspects, besides conservation characteristics. During the last three years we were debugging our database, and recently we published online a metadata regarding 12500 botanical registries, corresponding mainly to Andean taxa over 2000 m of altitude (75%). We could run geographical and taxonomical representativeness indexes at different scales and we could evaluate the curatory level of our collection and establish the priorities for the next years. We want to remark that the processes of database systematization and metadata update are meticulous and are continually bound to collection increase. Those activities have been made under the lineaments of a forerunner national program (SIB, Biodiversity Information System), which intend to divulge the information about biodiversity in Colombia, with the collaboration of several institutions with biological collections.

P2688. Web Services for distributed access to species checklists: Spice and Litchi

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The 'Spice' protocol provides a simple, lightweight, open and easily implemented communications channel, and is available to the biodiversity community for distributed read access to taxonomic and species-checklist databases across the Internet. It is currently used to connect source databases to a central hub and thence to a web-based user interface. It is being implemented on more than 30 databases around the world, each contributing one or more taxonomic sectors to the Dynamic Checklist of the Species 2000 and ITIS Catalogue of Life. The present platform- and programming language-independent HTTP/XML-based system implements a 'Common Data Model' at the species level. Web Services under development provide better support for higher taxa and taxonomic hierarchy browsing, and can link databases with alternative taxonomies and hierarchies. Compatible software implemented so far includes the Spice hub and web interface, a 'Wrapper Development Kit' to ease the task of connecting databases, and the 'Litchi' software to manage conflicting taxonomic concepts.

P2689. Types of Linnaean Plant Names Online

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The aim of the Linnaean Plant Name Typification Project is the completion of an annotated guide and catalogue to the typification of all valid plant names described by Carl Linnaeus between 1753 and 1776. The core of the Project is its database which holds information on all 9,127 Linnaean names validly published at the ranks of species and variety and including, for the names that have not yet been typified, data on the original specimens and illustrations used by their author. Many of these data are now freely available via the Project website. The site is fully searchable and allows users to obtain information on every individual binomial, including the original place of publication, the type and the author and place of publication of the typification, its current name, and any additional information relevant to its typification or application. Good quality images of type specimens from the George Clifford, Paul Hermann and John Clayton herbaria (all BM) accompany the species records and more images of type specimens and type illustrations are being added as and when they are available.

P2690. Online databases - global resources at the Natural History Museum, London

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Natural History Museum, London, UK, London, United Kingdom.

The Natural History Museum is a major repository of natural history collections made throughout the world and over a long period of time. Its earliest botanical collections date from the late 16th century. Conscious of the need to make these collections more widely available, the Museum has undertaken an ambitious programme aimed at increasing the accessibility of its collections. In recent years, the herbaria of Sir Hans Sloane, John Clayton, Paul Hermann and George Clifford, all rich in type specimens, have been digitised and the images made freely available online, with searchable databases to allow easy retrieval of information.

Currently, the Museum is actively involved in the Mellon Foundation funded African Plants Initiative. This aims to make available online, digitised and databased collections of all the world's African plant type specimens, along with associated library materials. We are also expanding such work and collaborations to include, for example, additional historical collections, cryptogamic plant groups, and Spruce specimens from Latin America.

P2691. TRGP (Taxonomic Repertory of the Genus Peperomia) & PBO (Peperomia Bibliography Online)

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It will be illustrated that CAT (Computer Aided Taxonomy) does not just mean Phylogeny Analysis and Interactive Keys. Taxonomists spend countless hours in gathering & accessing reference data. Tools optimizing these activities save a lot of 'unproductive' time and will, more and more, be part of every botanist's basic toolset... To study a particular taxonomic group, someone needs at least an up to date list of already published names, including synonymy annotations, references to the protologues and other publications, easy access to the type specimens and information about the distribution of the concerning taxa. *Peperomia*, with more than 3000 published names, is an interesting case genus. Data and digital images about this genus are online for almost five years now and the project has reached a sufficient stage of maturity. In addition, the TRGP and the PBO are used in several ongoing projects and additional features are added regularly. Existing repertories can easily be used as building blocks in 'tree of life' projects.

P2692. Extending access and opportunity in a world of varying electronic wealth: a demonstration based on the Web site for the Manual of Grasses for North America

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Web sites can provide access to a wide range of floristic information, but they are of limited value to those without easy Internet access. The *Manual* project is making more information available at its Web site than will appear in the printed volume. As a first step in making the resources more widely accessible, we are re-organizing the site (programs, documents, images, etc.) into a largely self-contained, stand-alone Web site, one that can be saved to a portable hard drive. A script will be written that makes installation of the stand-alone *Manual* a simple procedure. Updates to the file will be provided on a regular basis. This procedure has

the potential for expanding access to the project's resources to any botanist with a computer having extra hard disk space (~1GB), a Web browser and a Java Virtual Machine installed on a Linux or MS Windows platform. The files will not be free, but we shall try to minimize the cost. We are also planning to expand the site's scope through collaboration with taxonomists at other locations. Forms will be created for submitting information and resources via the Web or disk. The forms will be included in the stand-alone version of the Web site.

P2693. The Botanical Node within the German GBIF Network

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The aim of the Global Biodiversity Information Facility (GBIF) is to make the world's biodiversity data freely and universally available via the Internet (www.gbif.org).

The German GBIF node for Botany is coordinated by the Botanic Garden and Botanical Museum Berlin-Dahlem. The partners in the project work towards GBIF's goals by setting up an Internet portal for botanical collections and databases in Germany, integrating existing databases and networks, digitising herbarium collections, and jointly presenting them in a "Virtual Herbarium".

By means of BioCASE network software (protocol, wrapper; see www.biocase.org) and the ABCD (Access to Biological Collection Data) XML standard for data transfer, botanical databases containing about 2.7 million records have already been connected to the GBIF network. They are accessible via the international GBIF portal, as well as via the German GBIF Portal of Botany, which is focussing on German biodiversity and uses an advanced concept-based approach to access the data via taxon names.

P2694. Linking the past to the future: the AlgaTerra Information System

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AlgaTerra is a database, providing information on micro algae (especially diatoms and green algae) on the Internet (www.algaterra.org). The main aim of the AlgaTerra project is clarifying the usage of a name and adding referenced factual data to taxonomic concepts thus making historical knowledge fit for the Internet age and future research. AlgaTerra is an implementation of the "Berlin Taxonomic Information Model" (www.bgbm.org/biodivinf/docs/bgbm-model/) which was supplemented by a module to administer data on nomenclatural types. Illustrated types serve as calibration tool for related taxonomic concepts, which are linked to factual data, such as sequence data, morphology, photographs, ecology, and occurrence. The pilot phase of AlgaTerra (until 2005) was a joint project funded by the German Federal Ministry of Education and Research, merging the research data of five groups (BGBM, Berlin; Lazarus, Ehrenberg Collection, Berlin; Medlin, AWI Institut, Bremerhaven; Friedl, SAG, Göttingen; Reisser, Universität Leipzig).

P2695. The world's first virtual plant study trails as a tool for education, ecotourism, and vegetation research

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With partial support from Quality Education Fund (Hong Kong), we have established three virtual plant study trails. We started by taking serial digital photos of the vegetations along each trail and stitching these photos into a continuous panoramic photo, which is then turned into continuous flashes. These flashes were then loaded on our website <<http://hkflora.com/qef/index.php>>. Visitors can visit the virtual trails via the internet through a personal computer and scroll along the flashes to view the vegetations as if walking along the trails. When the mouse pointer touches a sensitive zone, the name and other information of the corresponding plant will appear. These virtual plant study trails will serve as useful tools to assist students in fieldwork trainings in botany and to assist guides in leading ecotours. The flashes have also captured the images of the vegetations along the trails at a particular time frame (i.e. 2003-2005 for our three trails) and will serve as *in situ* data of the vegetation at that time. Comparisons with future images of the same trails will demonstrate the

vegetation changes that have taken place between two time frames.

P2696. Taxonomic identification with standardized descriptions

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Up to now, many models to represent taxonomic information have been developed, but in spite of representing the same concepts, they don't exactly match. That's the reason because the IUBIS-TDWG is working on the development of a XML-based standard to allow a lossless exchange of information between different authors and different applications. This work describes a system to support the taxonomic identification process and includes the new requirements related to the intercommunication between representation models. That's at this way because is based on the new XML-based standard. The system has been developed following an expert system model and architecture, includes Artificial Intelligence techniques and it can be accessed over the internet. It has passed the verification and validation stage and the achieved experimental results endorse the suitability of these methods.

P2697. A comparative research about division rules to automatic generation of identification keys

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The automatic generation of identification keys can be tackled from a entirely computational point of view. Taking into account this approach, a key is equivalent to a decision tree (a graph with tree structure) which is generated from a set of examples, each of them belongs to a particular class (the taxa the key pretends to identify). Nodes will be the identification characters and leaves will be the objectives. The algorithm for generating trees divides the examples using a heuristic division rule which advises about the best character for each step of the key. The final key is different depending on the selected division rule, so we can obtain a great variety of keys from the same data set. This work presents a quantitative and qualitative research that analyzes the set of keys obtained applying different division rules over the same dataset. This research has taken into account well known division rules both in the Computer Sciences and Taxonomy area.

P2698. The web page of the vegetal biodiversity for the Douro Region (NE of Portugal)

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The elaboration of a dynamic web page about the natural vegetal resources of the Heritage Douro Region is here presented. The analysis carried out, which takes into account the importance of the biodiversity for the sustainable management of the area examined, involved a methodological process based on the correlation of the catalogue of the vascular plants and biophysical parameters. The data derived from this analysis were then used to propose a management for the vegetal resources that leads to sustainable development mechanisms. To ensure the widespread of the information derived from the present study among the scientific community a dynamic web page was elaborated. In this web page databases, georeferenced maps (type of soils, climatology variables, exposition, altitude, *), photographs (photographic records per taxon), ecoroutes (routes selected to observe diversity included in the database files) and information about the vegetal biodiversity (floristic catalogues) are found. The user of this web page will access to the different files included in whole the files of the database simultaneously.

P2699. Developing an Indian Botanic Garden Network for Conservation of Biodiversity

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BGCI has launched an international programme Investing in Nature in Argentina, Brazil, China including Hong Kong, India, Indonesia, Japan, Middle East, North America (including Canada

and USA) and South-East Asia. Under IIN-India project, an Indian Botanic Garden Network (IBGN) has been established to encourage a greater collaboration and sharing of resources (database) and knowledge among the botanic gardens in India; facilitate exchange of information through website, Newsletter, etc. and to create awareness among public to adopt better garden management practices. This paper discusses various aspects of the first of its kind IBGN database including data standards, methods of data collection, and searchable options on intranet and Internet (www.ibgn.org). IBGN database contains information of botanic garden resources of ca 60 Indian botanical gardens divided into six zones of India effectively representing the breadth of gardens both geographically and administratively.

P2700. Flora KwaZulu-Natal: making an electronic regional flora.

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The main aim of a local flora is to help non-specialists to identify the plants of the area covered. In the case of Kwazulu-Natal (South Africa), this aim can best be achieved by preparing the flora in both electronic and hardcopy forms. Thus the needs of users both with and without access to computers are met. The most effective way of doing this is to generate the electronic form first, and to use both old and new software to generate text for hardcopy from the electronic key files. The electronic version is generated in Lucid3. In this version, all character states used are illustrated, and all taxa included illustrated by (ideally) five or more photographs showing important features. An associated HTML page displays the text of the paper flora for each taxon, and allows space for additional information such as specimen citations and notes on common names, uses etc. The overall plan of the flora is discussed briefly.

P2701. Tela-Botanica: a network for francophone botanists

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Tela-Botanica is a network of botanist currently including more than 3000 members and around 40 discussion groups. It hosts the synonymic index of the French flora including three overseas départements (Réunion, Guadeloupe and Martinique) (<http://www.tela-botanica.org/eflore>). This nomenclatural dataset is based on the work of Michel KERGUELEN (1928-1999) and was converted into a database by Benoît BOCK. It was modified following thousands of comments and corrections by members of the network. The third version of the database includes 79930 names for 15799 taxa (a fourth enlarged version is to be launched in 2005). For each name is recorded the correct Latin name, place and date of publication and vernacular names.

Other documents are available on line : a French translation of the International Code for Botanical Nomenclature, a database of French Herbaria (with herbaria missing from Index Herbariorum), a database of publications on botany (25 000 articles), and lists of plant species for 70 départements (out of 96).

The network currently develops, among other things, tools for floristic inventories on PDA, and electronic methods for plant identification.

P2702. Basidiomycetes as the important component of the global biological resources

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Basidiomycetes include 32 orders and 140 families of agaricoid, aphylophoroid and gasteroid macromycetes. They are approximately estimated as 30% of known species of fungi. More than 3500 Basidiomycetes species are conserved and maintained in the world culture collections. Tens of edible and biotechnically significant species are cultivated for fruit bodies, natural products and enzymes using various types of wastes. Different drugs, dietary supplements and sanative beverages are prepared using Basidiomycetes. The researches on Biodiversity Conservation *ex situ* increase representation of genetic resources of Basidiomycetes in the culture collections and make them more accessible to users. Nowadays Basidiomycetes are included in the Global Biological Resources as a very important reproducible

component, which will have a positive impact on long-term food nutrition, health care and environmental conditions of people life.

P2703. Treatment of synonyms in specimen databases using web resources

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There is a large amount of information in a herbarium which must be efficiently managed so that it may be studied and knowledge obtained from it. There is therefore an evident need for information systems to fully manage both the information and the very functions of herbariums (Information administration, consultation processes, loan control, management of multimedia information, user administration, Report creation, Issuing of labels, etc.)

Nowadays, the main problem in the specimen databases included in these systems is the existence of synonyms which prevent studies and tasks from being carried out adequately.

In this paper, we shall present a tool which when included in classical herbarium management systems enables the verification of information about taxonomic names by incorporating other sources such as Anthos, Species 2000 and GBIF.

The services of tool are managed by means of a Multi-Agent System, i.e. an Input/Output system, which interacts with the web server.

P2704. National Database of DNA Profiles and RDA Integrated Information System (RIIS) of Plant Genetic Resources in R. Korea

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Application of biotechnology to genebank accessions for developing high through-put DNA profiling system was conducted in the Rural Development Administration (RDA) genebank of R. Korea. SSR (Simple Sequence Repeat) markers have been successfully developed in under-utilized crops through enriched method using magnetic beads. Target crops include rice, soybean, barley, wheat, corn, perilla, sesame, apple, pear, job's tear, mungbean and buckwheat. So far, 16,400 accessions of above crops have been profiled using 10 - 20 SSR markers and consequently subjected to a new database of DNA profile. Also the RDA Integrated Information System (RIIS) of plant genetic resources for providing DNA profile and other related data including passport, characterization, evaluation, seed image and geographical location of collection site was developed based on Internet assessable system. Web-based genetic analysis using characterization and DNA profile data is available and compounding survey of specific purpose for accessions is feasible by germplasm users through internet system. More details of RIIS will be introduced with the activities of RDA genebank.

P2705. Digitizing the *Flora Graeca*

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Oxford holds a wealth of material relating to Sibthorp and Smith's *Flora Graeca*, illustrated by Ferdinand Bauer and often described as 'Oxford's finest botanical treasure' - published 1806-40 and considered the most splendid and expensive Flora ever produced. A new digitization programme, scheduled for completion in 2005, will offer online access to the printed volumes, of which only 25 copies were produced, and to manuscript material hitherto only accessible in Oxford: the original hand-coloured drawings from which the printed engravings were made, unpublished drawings of the *Fauna Graeca* and a unique series of topographical *Mediterranean Scenes*, of great historical and geographical as well as artistic interest. This paper highlights the issues raised by the project, the methodology employed and the metadata captured, and evaluates the lessons learned. The project forms part of the developing *Oxford Digital Library* (www.odl.ox.ac.uk).

P2706. Index Hepaticarum : final publication and a new on-line database

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Index Hepaticarum (IH), a list of effectively published hepatic names, was started by C.E.B. Bonner at Conservatoire et Jardin botaniques - Geneva (G), in the 1950's. After his death in 1976 the

International Association of Bryologists took over the responsibility for completing the work. Bischler (PC) and Geissler (G) acted as editors for the new project, basing it at G for continuity. *IH* presents all published hepatic names citing the publication and protologue information, and the nomenclatural status for each. *IH* editions from G cover publications from 1 May 1753 to 31 Dec 1973. Names published after 1973 are collated as *IH* supplements by Engel and Crosby. The entry of names for the final section, *A-Jubula*, is now completed (10,000 entries). This work continues with verification of protologues and name validity. Our project database, soon available on-line, contains 30,000 hepatic names, all those published between 1753-1973. The publication of our final edition of *IH* and the debut of our searchable database will complement the work already done on the supplements to *IH*. Collectively this will be an enormous resource for the study of hepatics worldwide.

P2707. New digital life of old palynological collection.

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The palynological collection in Komarov Botanical Institute has been started at 1960 when our Palynological Group was established. Collection was permanently increasing in volume and till 1993 it consisted of more than 12000 specimens. In time we faced with some problem of maintenance collection in a good condition. The main were rather short life term of standard pollen grain specimen and impossibility to renovate most unique of them due to lack of herbaria materials. Since the beginning of 90th collection began grow shorter.

To prevent disappearance of the unique specimens and to make them accessible for specialists worldwide we made an attempt to create digital version of the collection.

For these purposes we have developed PostgreSQL database (DB) containing micrographs and morphological data of collections' pollen grains. PHP-powered DB user interface provides search for taxonomic and/or morphological characters.

Currently we are working to organize access to the DB via Internet.

P2708. Managing the Digital Revolution

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As other speakers in this symposium have made clear, there are many challenges for librarians and information specialists arising from the increasing trend towards digitization of collections as a means of making them available to a world-wide audience. There is great scope for collaboration between organizations holding split or complementary collections to enable better use of available resources and increase the collections' value to researchers, but this often involves international and cross-sectoral working which can be difficult to administer financially. Many decisions need to be made across disparate institutions at the initial planning stage for projects to succeed, in the areas of compiling resources, providing access, preservation and funding. This panel discussion will explore real-world practical solutions being embraced by panel members, and encourage audience participation in identifying user's needs and expectations which should drive future developments in providing virtual access to global resources.

P2709. An Interactive Key to Australian Orchid Genera.

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The Interactive Key to Australian Orchid Genera is an identification and information system for 190 genera of Australian orchids. Using Lucid software this powerful tool provides an easy to use and simple means of naming an orchid using any information the user has available. A total of 146 characters cover various aspects of the plants morphology as well as ecological and distribution information. The interpretation of the character information is assisted with help notes, colour photos and line drawings.

Included in the key are HTML fact sheets for each genus featuring botanical descriptions, common names, synonyms, geographic and ecological information, as well as natural history notes, distinguishing features, distribution maps and a comprehensive

references list. Each genus is illustrated with line drawings and colour photos and for some genera microscopy images are also included.

The Key to Australian Orchid Genera is a complete catalogue of information and an identification tool in one source. It would be a great benefit to a wide range of users from the professional researcher to those with the most basic botanical knowledge.

P2710. Green boon - A computer based multimedia compact disc for medicinal plants of India

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India has always been treasure trove of medicinal herbs. Though we have very old and strong traditional medical system we are unable to capture the world of herbal medicine in the true sense. Green boon is a pioneering effort to bring some parts of our vast knowledge on medicinal plants for your easy reference. It is friendly enough for the casual user who wants to gain some insights in to this wonderful herbal world. This computer based interactive CD is indeed a storehouse of invaluable information. It offers information on medicinal plants at your finger tips. Information is available on simple and easy to use format. Package contains quality images of five hundred medicinal and aromatic plants with descriptions and video clippings. Topics like biodiversity, biotechnology, herbal medicines, conservation etc are also explained. A value addition in the form of traditional knowledge related to medicinal plants is also provided. Details of how to cultivate selected medicinal plants are also given. Exhaustive index for easy navigation is also given. This is for the first time an interactive multimedia CD developed for the common man

P2711. Moving botanical illustration into the digital era

N. Simpson;

Independent research, part funded by Queen Elizabeth Scholarship Trust, United Kingdom.

A core component of systematic botany is the description, or re-description, of taxa. The accurate and measured composite plant portraits of botanical illustration form a valuable visual aspect of such description. The advent of electronic publishing and instant online access by an international audience has created new options for botanical illustration work.

This study (July 2003-August 2004) involved an investigation into the use of digital illustration techniques in providing the technological benefits of speed, accuracy, convenience and flexibility, without compromising the artistic values and clarity of traditional botanical artwork.

Results show that correctly designed and constructed digital illustrations can now provide fully realistic, accurate and comprehensive visual plant descriptions. Such digital illustrations are ideal for online or electronic access and their potential, for science and education, is immense.

P2712. The German GBIF Node for Mycology

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The international initiative "Global Biodiversity Information Facility" started in 2001. Already two years later the German GBIF Node for Mycology (www.gbif-mycology.de) was installed as service centre for mycological and lichenological biodiversity data in Germany. Its goal is to facilitate the online access to high quality data collections especially from ecologically and commercially important organisms. Meanwhile, the node is administrating a server network, acting as GBIF data provider, transferring data from proprietary databases into applications of the biodiversity component framework "Diversity Workbench" and is giving technical as well as scientific advice to database custodians. The German GBIF Node for Mycology is developing web clients and database wrappers for online access and is building up a sustainable IT infrastructure. Database applications are developed within the concept of the Diversity Workbench (www.diversitycampus.net/Workbench/download.html). The German mycological network originally started with 9 partner institutions and 11 data projects. Currently, 16 databases and information systems are associated.

P2713. Reducing the pain of digitising herbaria and sharing data.S. D. Grant¹, J. Wall², C. K. Frazier³;¹The Natural History Museum, London, United Kingdom, ²The Royal Botanic Gardens, Kew, London, United Kingdom, ³University of New Mexico, Albuquerque, NM, United States.

The drive to create digital herbaria has increased, as has the number of systems for doing so (eg the Berlin model, BG-Base, Biota, Biotica, BRAHMS, EMu, Herbar, Specify, Tropicos, and home-grown databases).

There are many complex, high-level standards such as ABCD, Darwin Core, HISPID, Linnean Core and the application of 'concepts', many of which concentrate on data exchange and not the actual recording of specimen information.

So starting up virtual herbaria can be difficult and make it seem better to create one's own system leading to difficulties in reliably sharing datasets with other institutions.

There appears to be a need for:

- 1) a set of guidelines describing the relationships between specimen data elements between data models;
- 2) practical examples about field contents;
- 3) practical guidance on database documentation.

The aim of this poster is to:

- 1) present a simple diagrammatic representation of specimen data using the historical record approach;
- 2) propose a workshop which will identify common elements within currently available data models, establish practical guidelines and collate real world examples on how to digitally represent specimens.

P2714. National Database of DNA Profiles and RDA Integrated Information System(RIIS) of Plant Genetic Resources in R. Korea

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Application of biotechnology to genebank accessions for developing high through-put DNA profiling system was conducted in the Rural Development Administration (RDA) genebank of R. Korea. SSR (Simple Sequence Repeat) markers have been successfully developed in under-utilized crops through enriched method using magnetic beads. Target crops include rice, soybean, barley, wheat, corn, perilla, sesame, apple, pear, job's tear, mungbean and buckwheat. So far, 16,400 accessions of above crops have been profiled using 10 - 20 SSR markers and consequently subjected to a new database of DNA profile. Also the RDA Integrated Information System (RIIS) of plant genetic resources for providing DNA profile and other related data including passport, characterization, evaluation, seed image and geographical location of collection site was developed based on Internet assessable system. Web-based genetic analysis using characterization and DNA profile data is available and compounding survey of specific purpose for accessions is feasible by germplasm users through internet system. More details of RIIS will be introduced with the activities of RDA genebank.

P2715. The availability of historical photographs - a pragmatic approach

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Herbaria, museums and other scientific institutions are often housing hundreds and thousands of picture negatives, reprints or colour slides. Unfortunately these pictures are often inaccessible due to several reasons. Sometimes there is no information available on the picture content or is difficult to access, e.g. as hand- or typewritten index cards.

Especially glass plate negatives are very fragile and in different condition states. The gelatine coating is often damaged or is not anymore firmly attached to the glass or even the glass plate is broken.

Despite these damaged objects, digitizing pictures and database storage of all available information will help to make this forgotten resource of information available to the scientific community. Biographies of researchers and photographers or itineraries of expeditions may complete the pictorial information.

A detailed workflow on preservation and digitizing historical images is presented and the output of our project on digitizing of historical images is shown on <http://bilder.botanik.univie.ac.at/>.

P2716. Balaton - A Century later of the Borbás' MonographyI. L. Szabo¹, P. Szeglet¹, K. Csermak¹, A. T. Szabo², P. Pomogyi³, Z. Domotorffy⁴;¹Georgikon Faculty, Keszthely, Hungary, ²Veszprem University Faculty of Teacher's Training, Veszprem, Hungary, ³West-Transdanubian Water Authority Department Kis-Balaton, Keszthely, Hungary, ⁴DigiScience Ltd., Pilisborosjeno, Hungary.

This study presents a comparative analysis of the researches done 100 years ago and today in connection with Lake Balaton.

Balaton proved to be the most intensively investigated lake at the beginning of the 20th century as a result of the "Issues of the scientific researches of Lake Balaton" program. The volume about the plants and phytogeography was composed by Vince Borbás and today it is regarded as a literary masterpiece, a basic botanical and phytogeographical discovery. Actual characteristics of the lake are significantly determined by the urbanisation, eutrophication and the recent years of drought. The previous minerogen and organogen succession has been replaced by ruderalisation. The lake bed sedimentation is very strong. There are spreading invasive neophytes impairing the archeophytes. The condition of the reed stands is grave. The above-mentioned phenomena and processes are represented and digitally demonstrated by the census and mapping of plant species and habitats in 2004.

P2717. The first ethnobotanical field book on internet: stirpium nomenclator pannonicus of St. Beythe (1583), Carolus Clusius (1583, 1584), D. Zwitteringer (1711) and F. Wolkingner et al. (1992)F. Wolkingner¹, I. Szabo², A. T. Szabo³;¹Institut für Pflanzenphysiologie der Universität, Graz, Austria,²Department of Botany, Georgikon Faculty, University of Veszprém, Keszthely, Hungary, ³University of Veszprém, Institute of Biology, Department of Botany, Veszprém, Hungary.

Stirpium nomenclator pannonicus is the first printed book on field ethnobotany edited by Carolus Clusius and Stephanus Beythe in 1583 in Güssing/Nemetujwar and reprinted later repeatedly as a document of early integration of Pannonian life sciences in European herbalism. Clusius was an early "Pan-European" botanist connected to the history of botany in Austria, Belgium, Croatia, England, France, Germany, Hungary, Italy, Slovakia, Slovenia, Spain, Switzerland, and especially The Netherlands. An Internet version of this valuable botanical field work is presented here, mirroring electronically the common botanical knowledge registered in 16th century Pannonia. The E-version analyses critically all the previous editions, making available worldwide a lasting document of European Herbalism and that of plant diversity explored and used by different ethnic groups of Pannonia about 420 years ago.

P2718. Present status of Atlas Florae Europaeae

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Atlas Florae Europaeae (AFE) is a program for mapping the vascular plant flora of Europe on the grid basis. It was launched in 1965, and thus far 13 volumes have been published, containing 3 556 maps of 3 276 species and 1 151 subspecies, and they cover more than 20% of the European vascular plant flora. The mapping work is performed by collaborators in all the European countries. New software, "Atlas Florae Europaeae Data Editor", has been developed to provide the mapping data electronically. From vol. 13 onwards a new grid was adopted. It is based on UTM and MGRS and extends over land and sea in a regular pattern. Total number of cells with land in Europe is 4 750. The old data has been converted into the new grid, and at present the AFE database comprises ca. 785 660 distribution records. The new grid is also used by several faunistic mapping projects in Europe and because of its regular pattern it is relatively easy to make co-ordinate converting programs from national grid systems to the new European grid. Besides printed Atlas volumes, the maps are shown online on the AFE web pages which facilitate updates of old maps, and the data itself has been used in various research projects.

P2719. Hungarian botanical monographs in the electronic ageL. Gerely¹, G. Borbély², L. Vörös³, A. Borhidi⁴;¹Biological Institute, University of Pécs, Pécs, Hungary, ²Biological Institute, University of Debrecen, Debrecen, Hungary, ³Biological and

Limnological Institute of the Hungarian Academy of Science, Tihany, Hungary, ⁴Institute of Ecology & Botany, Hungarian Academy of Sciences, Vácrátót, Hungary.

The following Central-European monographs written by authors of the Hungarian Taxonomical and Geobotanical School: are presented: Jávorka S., 1925, *Hungarian Flora*; Soó R., 1927, *Die geobotanische Monographie von Kolozsvár*; Jávorka S., Csapody V., 1929-1934/ 1975, *Iconographia florae Hungariae*; Nyárády E.Gy., Soó R., 1941-1944, *Flora von Kolozsvár*; Soó R., *The Phylogenetic System of Plants*; Soó R. 1964 - 1980, *Synopsis Systematico-geobotanica-Hungariae*; Borhidi A., O. Muñiz 1983: *Catálogo de Plantas Cubanas Amenazadas o Extinguidas*; Borhidi A., 1991: *Phytogeography and Vegetation Ecology of Cuba*. Borhidi A., 1995: *Phylogenic System of the Flowering Plants*; Borhidi A., 1996: *Critical revision of the Hungarian Plant Communities*; Borhidi A., Sánta A., (ed.) 1999: *Red Data book of the Hungarian Plant Communities I-II*; Borhidi A., 2002: *The Green Dress of Gaia*; Borhidi A., 2003: *Plant Communities of Hungary*. The E-presentation of a monograph is structured on the web (<http://bint-biotar.vein.hu/Collecta/Clusiana/IBC-2005>) as follows: Author selection; Title selection; Subject selection (Table of content); Text (doc/html/jpg); Comments; Miscellanea (PP Presentations).

P2720. Paul Kitaibel's descriptiones et icones plantarum rariorum hungariae (1802-1812) 2004 - and the electronic age

J. Géczi;

-, Budapest, Hungary.

Paul Kitaibel's (1757-1817) descriptions and pictures of the rarest plants of Hungary (*Descriptiones et icones plantarum rariorum Hungariae*) are a magnificent early result of botanical iconography and plant taxonomy of the Carpathian Basin. The work was sponsored by Francis Waldstein and edited in Vienna between 1802-1812, in three folio volumes (28 booklets) following the model of N. J. Jacquin's work, *Icones plantarum rariorum*. The unfinished series of *Descriptiones et icones* contains 280 hand coloured and scientifically perfect plant pictures. Only about 200 copies have been produced from this first Austro-Hungarian botanical iconography, with only 40 copies distributed in Hungary. Thirty copies were posted to England, but they all perished during the shipment; an additional 53 copies were confiscated by French authorities. Copies are also in Austria and in other countries, as well. The reprint of *Descriptiones* was published in Hungary in 2004, with sample pages of the iconography made available on the internet. The poster provides information about history of the work, together with its cultural and botanical values.

P2721. Sharing pictorial information via internet - a project based (mainly) on open source

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Sharing information combining visual and text based data is one of the most important skills in modern research. These informations are often maintained by large databases which are accessible via the Internet.

Despite professional databases like Oracle there are Open Source solutions which may help scientific institutions or communities to setup low-cost but powerful database solutions for the web.

Besides a low-budget system a user-friendly interface, a fast and manageable database administration system and a popular programming language is required for our purposes. A real classic combination is a Linux Server (SUSE, Debian, Red Hat, ?) incl. an Apache Web Server, a MySQL database and PHP (PHP: Hypertext Preprocessor - a recursive acronym) for the dynamic web interface. The challenge is now to combine input as well as text and graphical information both as visual and printed output. Design and workflow are shown in detail.

Check out our solution at <http://www.paldata.org/>.

P2722. Bibliographies and botany

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Bibliographies in any field may appear to be redundant in this era of the internet. Information professionals particularly the reference librarian or special subject librarians realize the importance of bibliographies in their daily encounters with clients. Has the

internet changed the composition of a quality bibliography? Does the botanical information on the internet act as a surrogate?

Taxonomic Literature second edition (TL-2) has become a standard and is a benchmark bibliography. Sadly, a great proportion of the literature for 'cryptogams' sensu Linnaeus is not included, leaving a major lacuna in the bibliographic tools for botanists working with these organisms. With the inspiration of TL-2 and the experience gained from building these databases we have embarked on a companion to TL-2, Taxonomic Literature Cryptogamia (TLC). TLC will be a descriptive bibliography based on primary sources, available in print and electronic media.

P2723. Testing databases of herbarium collections as a source of biodiversity studies

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Up to now numerous programmes and projects which promote and facilitate the processing of data of botanical collections have been made. We have developed our own computer system in the Herbarium of the University of Granada as the consequence of an interdisciplinary work between botanists and computer scientists. This system not only offers the computerization of our herbarium data and to take part in international initiatives such as GBIF, it also includes a series of Intelligent Agents which allow the evaluation of different parameters (geographical distribution, altitudinal distribution, ecology, phenology, etc) and biodiversity rates (*alfa*, *beta* and *gamma*) directly and accurately. We present this tool: BIOMEN (*Biological Management System Executed over Network*) in our poster and the results obtained through its application in the family *Cruciferae* Juss. of the province of Granada (Spain) represented in our collection.

P2724. Data capture and mapping priorities for herbarium specimens: putting conservation concerns at the top of the agenda

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Only about 3% of plant species have had their conservation status assessed following current IUCN criteria. To meet the targets of the Global Strategy for Plant Conservation (all known species to have preliminary conservation assessments by 2010) the rate of production needs to be increased massively. Analysing specimen data within a GIS can rapidly produce preliminary assessments and help address this need. We correlated conservation status of selected plant species with the number of herbarium specimens at three major plant diversity institutes. We found the repeated trend that the majority of threatened species had few specimens, and species with few specimens represented the majority of all species. The implications of this are that by databasing a relatively small percentage of the world's herbarium specimens, targeting those species represented by fewer specimens in key collections representative of the region concerned, we can obtain a preliminary assessment for the great majority of threatened plant species globally.

P2725. Biodiversity pattern and beyond: integrating herbarium-based informatics with phylogenetic and environmental data

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Australia's Virtual Herbarium, a collaborative national specimen digitisation and web-based dissemination project, is providing unprecedented opportunities to map patterns of the distribution of taxa and collective measures of diversity of the Australian flora. Through case studies we illustrate its potential, when integrated with phylogenetic and environmental data in geographical analytical frameworks, for conservation planning, macro-ecology and evolutionary biology. Conservation planning requires taxonomically correct, geographically representative information on species distributions. Analyses of the environmental distribution of herbarium records is providing insight into collection bias and the

scale at which diversity patterns can be reliably resolved to inform conservation policy. Analyses of the geographic and environmental distribution of monophyletic clades, that explicitly examine spatial scale and non-stationarity of patterns, are providing exciting opportunities to assess the relative roles of the contemporary environment and historical evolutionary processes in the distribution of gradients of diversity.

P2726. The role of herbaria and flora in preserving local plant-use information: the case of the Ethiopian national herbarium and flora

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The aim of this study was to compile and analyse information on local use of plants based on data obtained from labels of specimens stored at the National Herbarium and Flora volumes of Ethiopia. For the study, two families were considered: Fabaceae and Euphorbiaceae. Analysis of the data recovered from the specimen labels of the two families yielded a total of 116 locally useful plant species in Ethiopia. Medicinal use claimed the highest proportion of plants (52%). The study also revealed *Acacia nilotica*, *Croton macrostachyus* and *Ricinus communis* as having the highest use diversities. Further analysis of the herbarium data belonging to Euphorbiaceae demonstrated an increasing trend, with time, in the incorporation of plant-use information by collectors on specimen labels although much of the records were found incomplete to be considered sufficiently useful. The study also revealed that the bigger extent of use information given on herbarium labels regarding the two families was not incorporated in the relevant Flora volumes. All this justifies the need for carrying out proper ethnobotanical investigations or targeted collections in the country.

P2727. Modelling species palaeo- distribution patterns in BDWorld - implications for predicting the impact of global climate change

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Using state of the art climate model data covering the past 22 thousand years and present day specimen locality collections, we model the possible palaeo- distributions of a number of plant species. By direct comparison with pollen distribution records from the Global Pollen Database, the relationship between the actual palaeo- distribution and the modelled potential distribution in various time periods is analysed. Whilst there have been many previous attempts to model species distributions in present climatic conditions, the relevance of similar predictions for the future have yet to be fully verified. By using independent distribution data for the past in the form of fossil pollen, the relevance of static distribution modelling tools in temporal distribution modelling is therefore assessed for the first time. This high-throughput modelling procedure has been made possible through the BDWorld project utilising distributed GRID computing technologies.

P2728. BiodiversityWorld: A problem-solving environment for global biodiversity.

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The goals of the BDWorld project are to create an extensible framework for supporting distributed GRID computing in biodiversity research, with seamless access to remote data and analytical tools. From the users' perspective the system consists of a graphical work-flow manager that provides access to a rich set of remote data resources and analytical tools. From the system perspective there is a layered architecture with replaceable communications protocols, a workflow designer, a workflow enactment engine, a metadata repository for semantic mediation and reasoning, a resource locator, a resource matcher, and a suite of wrappers or proxies for the remote resources. We present an overview of the system architecture and development, and demonstrate examples of its use in three exemplar study areas: (i)

biodiversity richness analysis, (ii) bioclimatic modelling and climate change scenarios, and (iii) phylogenetic and biogeography analyses.

P2729. Identification key for the cypress family (Cupressaceae)

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The identification of Cupressaceae taxa, except for some local and easily distinguishable taxa, is difficult even for specialists. One reason for this is the lack of a complete key including all Cupressaceae taxa, another reason is that diagnoses and descriptions are spread over several hundred publications which are sometimes difficult to access. Based on morphological studies of about ¼ of the species and a careful compilation of the most important descriptions of Cupressaceae, a first interactive identification key for the entire Cypress family (Cupressaceae) could be set up. The key comprises any of the 30 genera, 134 species, 7 subspecies, 38 varieties, 1 form and thus all 180 taxa. The conventional key uses mainly features of adult leaves, female cones and other characters which are all relatively easy to be used. The key was published as a conventional key and digital key with more than 1000 detail-illustrations and a lot of additional information about common names, synonyms and features.

P2729. The natural habitats for edemic, rare and threatened species from the Abruptul Prahovean Reserve (Bucegi Massif, Southern Carpathians)

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Abruptul Prahovean is a natural reserve of the Bucegi Massif (Merridional Carpathians), on the southern slope of Caraiman and on the Jepsi valley. There is an area of strict protection exempt from grazing in this reserve. It has been constituted since 1955. In this area we emphasize the importance of a 45 endemic, rare and threatened plant species (Cormophytes) in the regional context. According to their statut in the Carpathians area the species are also included in Romanian Red Lists. However, there are many studies in this protected area but in this paper we selected this list of endemic, rare and threatened species and described them and their habitat types. More over, we selected the codes for them in accordance with the database from the EMERALD software; the habitat classification is based on "A classification of Palearctic habitats", Habitats Directives - 92/43/EEC and of Romanian Law 462/2001 Annex 2. Consequently, we used EUNIS code for habitats, related to phytosociological associations, as described in Romanian phytosociological literature.

P2730. Iranian medicinal plants monographs

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In the computer and information age, using new technologies for storage, retrieval and analysis of information is vital. Objectives of this work were documentation of published and unpublished literature on medicinal plants of Iran, to facilitate information exchange between scientific communities by digitalizing the monographs and to harmonize the information on medicinal plant and use of herbal medicine. Each monograph contains detailed information on botanical family and scientific name, vernacular name, botanical description including photos, habit and habitats, distribution maps. Detailed ethnobotanical information, medicinal uses, chemical composition and compounds, pharmacology and dosage have been included. A computer program has been developed to restore this information. This information is provided for 100 species up to now. These species belong to 39 families. This work is still in progress to cover all the medicinal plants used in Iranian Traditional and folk medicine.

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