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Biogeographic variations in wood mice: Testing for the role of morphological variation as a line of least resistance to evolution

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Abtract

Evolutionary studies of morphological characters often depict patterns of differentiation and interpret them as response to selection or random divergence related to the degree of isolation between populations. The pattern of differentiation, however, may also reflect intrinsic constraints related to genetic and developmental networks. How these constraints contribute to orientate evolutionary trajectories is nowadays a key topic bringing together evolution and development in an evo-devo perspective. Morphological variation in a population, being the product of genetic and developmental processes, can constitute a precious insight into such constraints. Widespread phenotypes being easy targets of both selective screening and random sampling, the direction of main variance could act as a 'line of least resistance to evolution'.

Extensive biogeographic variation has been described in the European wood mouse (*Apodemus sylvaticus*) in morphological characters such as the molar tooth. A latitudinal gradient has been evidenced on the continent, interpreted as an adaptive response to biogeographic clines in food resources. Extensive divergence is further documented on various islands, that could be attributed both to adaptation to peculiar insular conditions, and/or to drift related to isolation. Using geometric morphometrics, and based on this well documented biogeographic background, we explore here in what respect these different patterns of differentiation can be related to the directions of main intra-population morphological variation. Most striking cases of insular divergence, as well as the latitudinal cline, occurred parallel to the main direction of morphological variation. This validates its proposed role as line of least resistance to evolution favoring rapid morphological evolution, either as response to selection or as a result of drift or both. Whatever the process involved, this line of least resistance is likely to promote parallel evolution in various contexts.

Introduction

Morphological variation is an important aspect of biodiversity, in particular because phenotypic variation is an important target of the screening by selection. Its study can bring light onto the adaptive component of morphological diversification, thus constituting a precious complement to the vastly and rapidly developing field of genetic and genomic analyses. Furthermore, morphological evolution can be studied on both modern and fossil species, and can thus help to bridge the gap between different temporal scales, from contemporary evolution to long term trends along millions of years.

Patterns of morphological evolution have long been studied, including for deciphering rodent evolution (e.g. Misonne 1969; Michaux 1971; Butler 1985). This field of investigation has been renewed by the development of methods allowing the quantification of fine-scale shape variation, namely geometric morphometrics (e.g. Rohlf and Marcus 1993; Bookstein 1991; Mitteroecker and Gunz 2009). Such methods, based on landmarks or outline analyses, have been used to tackle many topics regarding rodent evolution: evolutionary patterns along fossil lineages (Renaud *et al.* 1996, 2005; Piras *et al.* 2009; Stoetzel *et al.* 2013), diversification among species, addressing the respective role of adaptation and neutral evolution (e.g. Cardini 2003; Monteiro *et al.* 2005; Macholan 2006; Michaux *et al.* 2007a); differentiation between populations, investigating the role of environmental variations (Renaud 1999; Fadda and Corti 2001; Renaud and Michaux 2003, 2007; McGuire 2010; Helvaci *et al.* 2012), processes favoring co-occurrence among species (Ledevin *et al.* 2012), patterns and route of colonization (Valenzuela-Lamas *et al.* 2011; Siahsarvie *et al.* 2012; Cucchi *et al.* 2013). Insular differentiation provided numerous models of pronounced morphological differentiation questioning the respective role of adaptation and random factors (Cardini *et al.* 2007a; Michaux *et al.* 2007b; Renaud and Michaux 2007; Renaud and Auffray 2010; Renaud *et al.* 2013). Even contemporary evolution and response to current anthropic changes can find a morphological signature in rodents (Pergams and Lacy 2008; Renaud *et al.* 2013).

All these studies rely on the characterization of patterns of morphological differentiation. Advances in evolutionary biology, quantitative genetics (e.g. Klingenberg *et al.* 2001; Workman *et al.* 2002) and developmental biology (e.g. Kassai *et al.* 2005; Kavanagh *et al.* 2007; Prochazka *et al.* 2010) challenged to bridge the gap from patterns to processes within an integrated 'evo-devo' framework. The analysis of morphological variation is of interest in this context (Polly 2008; Renaud and Auffray 2013) because it is the product of the underlying genetic variation and developmental pathways. Widespread variants can point to developmental mechanisms favoring their production (e.g. Renaud *et al.* 2011). They will have a higher chance of spreading within a population, either by selection or by

random processes such as founder effects and drift. Hence, directions of main variance have been proposed to constitute 'lines of least resistance to evolution' (Schluter 1996).

The concept was first developed for the direction of main genetic variance (Gmax) which is the principal vector of the genetic variance-covariance (VCV) matrix (G matrix). However, considering the phenotypic variance (P matrix and its main direction Pmax) presents several advantages. Phenotypic variance can be assessed from any set of morphological measurements on a sample of specimens, whereas estimating the G matrix requires known genealogies (Steppan *et al.* 2002). Pmax can thus be studied in wild populations (e.g. Ackermann and Cheverud 2000; Marroig and Cheverud 2005) and even in fossils (e.g. Renaud *et al.* 2006; Hunt 2007). G matrix and P matrix share most of their directions of variance (Siahsarvie 2012), suggesting that the latter can be confidently used as a surrogate for the former. Furthermore, compared to the G matrix, considering the P matrix incorporates developmental and environmental component of the phenotypic variation.

The direction of main phenotypic variance, Pmax, corresponds to a vector pointing in the direction of the most widespread variation. A first question is to know if this direction is conserved across populations and even species, a hint that the underlying genetic / developmental networks would be conserved along evolutionary time scales (Ackermann and Cheverud 2000; Bégin and Roff 2003; Renaud *et al.* 2006; Renaud and Auffray 2013). However, the structure of the variance itself can evolve due to selection or drift (Roff and Mousseau 2005). A second question is to know if the pre-existing variance actually tends to favor evolution into this direction. This can be addressed by comparing the direction of Pmax to evolutionary directions among populations or species (Marroig and Cheverud 2005, 2010; Renaud *et al.* 2006; Hunt 2007).

The aim of the present contribution is to illustrate the study of Pmax and its use for deciphering processes driving morphological diversification on the model of the European wood mouse (*Apodemus sylvaticus*). The first upper molar (Fig. 1) was selected as trait of interest because its evolution among modern populations and along fossil lineages of murine rodents has been evidenced to match such a line of least resistance to evolution, matching an overall conserved direction of main intra-population variation (Renaud *et al.* 2006; Renaud and Auffray 2013). This direction of shape change tends to oppose broad vs. elongated molars (e.g. Fig. 1).

Intricate patterns of differentiation have been previously evidenced in the wood mouse. Molar shape differences have been found among phylogenetic lineages (Renaud and Michaux 2007) inherited from repeated isolations in separate refuges during glacial-interglacial cycles (Michaux *et al.* 2003). Interfering with this differentiation between isolated populations, a morphological cline was

evidenced across Europe, possibly related to large-scale trends in food resources (Renaud and Michaux 2007). Finally, the wood mouse is an opportunistic species that accompanied human travels during prehistorical and historical times (Genovesi *et al.* 2009). It thus colonized many islands, where it displays cases of pronounced phenotypic evolution (Renaud 2005; Renaud and Michaux 2007).

The shape of the first upper molar (UM1) was quantified using an outline analysis method, allowing for the estimation of within- and between-populations patterns of variation. The following questions were then addressed. (1) Was Pmax conserved among populations? This was tested in a series of comparisons between populations: within lineage, among lineages, and in an insular population where adaptation and drift could have promoted local divergence. (2) Did Pmax channel evolution into favorite directions, validating its proposed role of 'line of least resistance to evolution'? This was tested in several situations: (2.1) among lineages, involving a probable role of drift between isolated groups; (2.2) along the latitudinal trend on the continent; (2.3) on islands, where adaptation and random factors may both promote divergence.

Material and Methods

Material

The study is based on a data set of 586 wood mouse first upper molars (Table 1). Most specimens were genetically diagnosed as *Apodemus sylvaticus*, discarding possible mixing with the related species *Apodemus flavicollis*. The only exception were specimens from the collection of the Museum National d'Histoire Naturelle (MNHN, Paris, France) and from the collection of the Centre de Biologie et Gestion des Populations (CBGP, Montpellier, France), but all originated from areas in Western France were *A. flavicollis* is not documented (Quéré and Le Louarn 2011).

Most of the wood mice considered belong to the Western European genetic lineage, including populations from mainland and various islands (French Atlantic Islands: Oléron, Noirmoutier, Ré; Mediterranean Hyères Islands, off South-Eastern France: Port-Cros and Porquerolles; Ibiza off Eastern Spain). The italo-balkanic genetic lineage was represented by localities from mainland Italy and various islands (Corsica, Sardinia, Elba). Sicily and the satellite island Marettimo correspond to a separate lineage related to the Italian one (Michaux *et al.* 1998b). North-African populations correspond to a separate lineage related to the Western European one (Michaux *et al.* 2003) but it is documented here by a single locality.

Sex of the specimens was known in three well-sampled populations (Mimizan, Lantabat, Tourch) in which the occurrence of sexual dimorphism in molar size and/or shape was investigated. The Lantabat sample was trapped in four successive seasons (two in spring and two in autumn) allowing the investigation of the influence of the period of trapping. The Mimizan sample was composed of a set of four places distant of few kilometers, and this allowed the investigation of possible local variations in a metapopulation.

All specimens had all their third molars erupted. Because the third molar erupts at the period of weaning, this is a classical criterion for excluding juvenile specimens. All animals were thus considered as sub-adults and adults.

Methods

Outline analysis of the first upper molar

Molar shape was estimated by the two dimensional outline of the tooth in occlusal view, with focus towards the basis of the crown, which is affected late by progressive wear during the animal's life.This molar shape quantification has been shown to be relatively insensitive to age classes in wood mice, except for very young or very old animals that are anyway rare in most wild populations (Renaud 2005). Such an approach has been shown efficient to evidence patterns of biogeographic variation in the wood mouse (Renaud 2005; Renaud and Michaux 2007).

Each outline was described by a set of 64 point at equal distance along the outline. The starting point was tentatively located at the anteriormost part of the tooth, and adjusted by aligning the starting point with the long axis of the tooth. Outlines were then analyzed using a radial Fourier transform (RFT) (Renaud *et al.* 2006; Renaud and Auffray 2013). Using this method, a set of radii (i.e. distance from each point to the center of gravity of the outline) was calculated from the original coordinates, and this set was decomposed into Fourier functions, i.e. trigonometric functions of decreasing wavelength termed 'harmonics'. Each harmonic is weighed by two Fourier coefficients (FCs) that constitute the shape variables. The zero harmonic (A0) corresponds to a best-fit circle to the outline. It was used as size estimator, and to standardize all other FCs for isometric size differences. This was performed by dividing all raw FCs by A0. Consideration of the first seven harmonics was considered a satisfactory compromise between information content and number of variables for both characters (e.g. Renaud and Michaux 2007). The 14 resulting FCs (two FCs per seven harmonics) were used as shape variables.

Statistics

Differences between groups (sexes, localities, geographic groups) were investigated using analyses of variance (ANOVA) completed by their non-parametric analogue (Kruskall Wallis test, KW) for univariate variables, and multivariate analysis of variance (MANOVA) for the set of shape variables.

The pattern of shape variation was assessed using a principal component analysis (PCA) on the variance-covariance (VCV) matrix based on the 14 shape variables (FCs). This provided synthetic axes (principal components (PCs), e.g. eigenvectors) summarizing the most important directions of variance within the data set.

Relationships between the set of shape variables considered as dependent matrix, and another quantitative variable (e.g. size or latitude) were investigated using multivariate regressions.

Directions of main variance and comparison of vectors

Pmax was calculated as the first eigenvector of a PCA on the intra-population VCV matrix of the 14 shape variables. Directions of evolution were calculated as the vector of difference between two populations (e.g., an insular population and its putative mainland relative) or as direction of main phenotypic variance among a set of populations (first eigenvector based on the VCV matrix among population means).

Similarity between vectors (Pmax and evolutionary directions) was assessed by calculating their correlation R, i.e. the arccosine of the inner product of the two vector elements, ranging between -1 (vectors pointing in totally opposite directions) and +1 (vectors perfectly pointing in the same direction). This observed correlation was compared to the distribution of R from fifty thousand simulated random vectors. Each random vector was calculated as follow: each of its 14 elements was drawn from a uniform probability distribution in the range from +1 to -1, and the vector was then normed to unity. The distribution of these random vectors provided the following significance threshold values for the absolute value of R (a significant probability meaning that the observed R is larger than expected based on the distribution of R between random vectors): $P < 0.01$, R = 0.651 (*); $P < 0.001$, R = 0.770 (**); $P < 0.0001$, R = 0.860 (***). Note that the absolute value of R was considered, because the +/- direction of Pmax (and of any eigenvector) is arbitrary.

In order to estimate the robustness of Pmax and of the evolutionary directions regarding sampling, each considered geographic group was bootstrapped 100 times. Pmax, allometric directions, and directions of changes between groups were calculated on these bootstrapped samples, and compared with the original direction. The mean correlation value provided an assessment of the stability of the vector regarding sampling.

Results

Test of sexual dimorphism and local variation

Sexual dimorphism was evidenced in none of the cases (Table 2), a result congruent with other studies on wild populations of wood mice (Renaud 2005; Ledevin *et al.* 2012) and domestic mice (Valenzuela-Lamas *et al.* 2011). Males and females were therefore pooled together in subsequent analyses.

Season of trapping in Lantabat was not related to any differences, supporting the idea that age structure of a population had no impact on estimates of molar shape. Differences between sub-localities in Mimizan were significant for molar size and close to the significant threshold in molar shape, indicating that variations at a small spatial scale might interfere with variations at larger scale.

Geographic patterns in molar morphology

Differences in molar shape were significant among groups (MANOVA: P < 0.0001). Mainland populations tend to scatter in two groups along the first multivariate axis PC1, with most localities being close to the origin but all Western French populations clustering towards positive PC1 values (Fig. 2A; for a visualization on a geographic map, see Fig. 3A). These populations share slender molar shape. Almost all insular populations scatter around this core mainland variation. Several islands (Hyères Islands of Porquerolles and Port-Cros, French Atlantic islands of Oléron and Ré, Southern Corsica, Marettimo islet off Sicily) display extreme negative values along PC1, corresponding to very broad molars (Fig. 2B). The Gambarie sample (southernmost extremity of mainland Italy) constitutes the only mainland sample clustering with these insular populations. Sicilian populations tend to isolate along PC2. This axis tends to opposes molars wide at their posterior part (positive PC2 values) to molars with a short and broad forepart.

Molar size also varied significantly across groups (ANOVA and KW: P < 0.001). According to previous results (Renaud and Michaux 2007), the main pattern corresponds to a trend of larger molars

towards Mediterranean regions (Fig. 3B). In few cases, insular populations tended to display larger molars than their mainland relatives, especially in Porquerolles. The trend observed in the islands of Yeu, Elba and Marettimo has to be taken with caution since these islands were sampled by a single specimen each.

Stability of Pmax among populations

The robust estimation of Pmax requires well-sampled populations (Polly 2005; Renaud and Auffray 2013). Pmax was thus evaluated in populations documented by more than 30 specimens (Table 1). Tourch, Mimizan, Lantabat and Tarquinia documented mainland populations, with two different phylogenetic lineages represented (Western Europe and Italy). Porquerolles further documented an insular situation.

Pmax was robustly estimated in all populations and highly correlated among them (Table 3; Fig. 4). The correlation was especially high (R > 0.9) between all mainland Pmax. Pmax in Porquerolles was less strongly correlated to mainland directions (0.7 < R < 0.9). Pmax corresponds to a trend ranging from stocky, broad molars to elongated, slender ones (Fig. 2B).

Pmax and evolutionary directions on mainland

The role of Pmax as possible channel to evolution was investigated by comparing Pmax to various evolutionary directions. The overall direction of evolution on mainland was estimated as the first axis of the inter-group VCV ($V1_{mainland}$; 30.6% of the variance). This direction, summarizing all other directions of variation, was sensitive to sampling. This may be due to the fact that it relies on a limited numbers of group means (N = 33). Furthermore, the limited amount of variance explained by this first axis lets much of variance on subsequent axes, and the first eigenvectors may be permuted in some bootstrapped samples. Nevertheless, this direction was congruent with Pmax of most populations (Table 4; Fig. 4).

The directions of evolution between mainland biogeographic groups (Western European vs. Italian lineages, and within the Western European lineage, Western France vs. continental populations at the same latitude) were further compared to Pmax (Table 4). These directions were not parallel to Pmax.

A latitudinal trend in molar shape was previously described in the European wood mouse (Renaud and Michaux 2007). It was confirmed here on the total mainland data set (multivariate regression of FCs vs. latitude: N = 413, P < 0.0001). The latitudinal trend in molar shape was parallel to all documented Pmax. Although discrete, it corresponds to a similar trend ranging from broad to slender molars (Fig. 2B).

Allometric variations

Significant molar size variations were documented across geographic groups. Trends in molar size may contribute to the pattern in molar shape if both are coupled by allometric mechanisms. Overall, intra-population allometry was very limited, being non-significant in three out of five populations (Lantabat: P = 0.461; Tarquinia: P = 0.206; Porquerolles: P = 0.085). The two significant intra-population allometric relationships (Mimizan: P = 0.013; Tourch: P < 0.001) were not robustly estimated, not related together and not related to Pmax (Table 5; Fig. 4).

The inter-population allometric direction was estimated on mainland, based on group means and on all specimens, both providing robust and similar estimates of the allometric direction (Table 5). These directions were not related to Pmax, and were not or only weakly related to intra-group allometry (Table 5). Overall, this suggests a limited role of allometry in molar shape differences.

Pmax and insular divergence

Neighboring mainland localities were considered as reference for evaluating the importance of insular divergence. All islands documented by a single specimen were not further investigated. Porquerolles emerged as well differentiated among the Hyères islands while Port-Cros was not (FR-SE vs. Port-Cros: P_{MANOVA} = 0.100; vs. Porquerolles: P < 0.001). Ibiza was not differentiated from mainland Spain (vs. SP-E: P = 0.080). Among islands related to the Italian clade, Corsica was weakly differentiated while Sardinia was clearly divergent (IT-CW vs. Corsica: P = 0.031; vs. Sardinia: P < 0.001). Among Atlantic French islands, Oléron and Ré were significantly divergent (FR-W vs. Oléron: P < 0.001; vs. Ré: P < 0.001). Noirmoutier molars were not significantly differentiated from the continent (P = 0.310). Finally, Sicily constitutes a peculiar case of an old endemic lineage, related to the Italian clade from which it significantly differ in molar shape (vs. IT: P = 0.010).

For the insular populations showing a significant divergence, the direction of differentiation was compared to Pmax (Table 6; Fig. 4). In all cases except Sardinia, the insular divergence followed the

direction of Pmax. Note that most islands such as Porquerolles, Oléron and Ré shared a trend towards broad molars (Fig. 2B). In contrast, Sicilian molars evolved parallel to Pmax but in the opposite way, with slender molars than the Italian stock.

Discussion

This study evidenced a complex pattern of molar shape differentiation in the European wood mouse *Apodemus sylvaticus*. Main features are: (1) the importance of morphological differentiation occurring in many islands, by far larger than any divergence on continent; (2) a weak but significant differentiation among the phylogenetic lineages, with an emerging clustering of the Westernmost French localities, not identified until now; (3) a latitudinal trend in molar shape. The corresponding directions of evolution were compared to the direction of main phenotypic variance Pmax, in order to assess its potential role in channeling evolution.

Stability of Pmax across populations

Pmax was evaluated in five well-sampled populations, documenting the two main phylogenetic lineages and one insular situation. Overall, Pmax pointed in the same direction whatever the population, supporting the stability of the underlying genetic / developmental networks over evolutionary time scales. This is in agreement with previous studies, showing a conservation of Pmax along the evolutionary lineage of the wood mouse, and across related murine lineages over more than 10 myr (Renaud *et al.* 2006; Renaud and Auffray 2013).

Among these correlated vectors, Pmax in Porquerolles appears as the most divergent. Porquerolles is a small island (~13 km²) few kilometers off the French Mediterranean coast. Inter-specific competition and predation levels are decreased on this islet (Renaud and Michaux 2007), factors that could promote fast adaptation. Effective population size is also highly reduced compared to the continent, favoring drift cumulated to initial bottleneck effect. Not mutually exclusive, regular immigration from the nearby mainland may lead to admixture biasing the pattern of intra-island morphological variance (Guillaume and Whitlock 2007). All these effects have been shown to cause evolution in the G and/or P matrix (Roff 2000; Cano *et al.* 2004; Roff and Mousseau 2005). Pmax in Porquerolles may document a case of local evolution in the structure of the phenotypic variance, promoted by reduced population size, despite the reduced genetic differentiation from neighboring mainland populations (Michaux *et al.* 2002).

Limited role of allometry in molar shape evolution

Size -related shape changes have been suggested, and indeed demonstrated as a line of least resistance to evolution in bony traits such as skull and mandible (Marroig and Cheverud 2005; Cardini and Thorington 2006). Such traits are prone to change along growth due to bone remodeling, making of intra-population allometry a significant component to Pmax (Renaud and Auffray 2013). In contrast, murine molar teeth are determined early during the embryonic development and are only affected by wear after eruption. This may make them less prone to allometric variations. Indeed, allometric directions of molar shape changes were not congruent between intra- and inter-population levels, were not parallel to Pmax, and did not contribute significantly to any of the evolutionary shape changes documented here. This undermines the role of allometry as a significant evolutionary force in traits such as murine molars, although allometric constraints may be of prime importance for other traits such as skull (e.g., Cardini and Polly 2013).

Divergence between phylogenetic lineages does not follow Pmax

Evolution on the continent and on islands may follow different regimes, due to very different conditions regarding population size, demography and degree of isolation. On the continent, large effective population size and possibility of gene flow between neighboring populations should tend to buffer variations and smooth morphological divergence. Nevertheless, morphological differences may slowly accumulate proportional to genetic divergence between isolated lineages (e.g. Renaud *et al.* 2007). In agreement, a significant molar shape differentiation was observed between the Western European and italo-balkanic lineages, separated since more than 1 million year (Michaux *et al.* 1998a; 2003). A further differentiation may occur within the Western European lineage, with clustering of the Westernmost French populations suggesting a phylogenetic relatedness that challenges further studies in this so far undersampled area. In both cases, the differentiation between these groups did not occur parallel to Pmax. Possibly, stabilizing selection may be an important component in the evolution of this generalist and opportunist rodent (Renaud *et al.* 2005), thus constraining evolution along Pmax. Mutually not exclusive, minor changes may still accumulate along other directions (Renaud *et al.* 2006).

Sicily represents in this context a peculiar case, since this island houses an endemic lineage isolated for more than 800 000 years (Michaux et al. 1998b). Ancient and intense human travels might have

partially counterbalanced the insular effect, tending to promote morphological divergence. Yet, in this case, the divergence between this lineage and its Italian relative occurs parallel to Pmax. The respective role of isolation and adaptation are difficult to disentangle in the case of this endemic lineage.

Latitudinal trend parallel to Pmax

Superimposed to the differentiation among clades, a latitudinal trend in molar shape has been documented in the European wood mouse. It corresponds to broad teeth towards the South and slender teeth towards the North (Renaud and Michaux 2007), echoing a trend in mandible shape found parallel in several species of wood mice (Renaud and Michaux 2003). As most clinal geographic trend, it has been interpreted as a response to selection (e.g. Cardini et al. 2007b), possibly related to variations in food resources across Europe. Such a response to a presumably moderate selection might be facilitated if occurring along Pmax.

Yet, the interpretation of any clinal variation as a result of the action of selection has been challenged, suggesting that isolation by distance among populations connected by spatially limited gene flow might produce similar clinal variations (Vasemägi 2006). Evolution along Pmax might equally facilitate the occurrence of a morphological latitudinal cline through such a process. By favoring morphological evolution along few directions, due to selection as due to random processes, the channeling role of Pmax makes difficult to disentangle both processes (Ackerman and Cheverud 2004).

Parallel evolution on islands: exemplary cases of the channeling role of Pmax

Compared to the relatively limited divergence between lineages separated since one million years, insular evolution displays amazing cases of molar shape divergence, as exemplified on Porquerolles, despite close genetic relatedness with wood mice from the nearby mainland (Michaux et al. 2002).

Insular divergence, however, is not systematic and several islands display no or weak differentiation (Noirmoutier, Port-Cros, Ibiza, Corsica). In most of these cases, these islands are densely populated, for some of them since antiquity (Vigne and Valladas 1996), and the frequency of human travels might have promoted exchanges among wood mice populations and dampen phenotypic divergence. In contrast, pronounced insular divergence was overall displayed on small islands (Porquerolles, ~13

km²; Oléron, 18 km², Ré, 9 km²), suggesting a key role of random processes, i.e. founder effect, bottleneck and drift, in promoting differentiation.

Yet, random processes alone should lead to differentiation on the various islands according to various directions corresponding to the directions of the P matrix matrix, leading to a similarity in the directions and eigenvalues of the P matrix and of the inter-group VCV (Ackerman and Cheverud 2004). In contrast, here, in all the cases of pronounced differentiation, evolution occurred in a parallel way. An adaptive component may be proposed: due to a decrease of interspecific competition, niche widening is likely to occur on many islands (Orsini and Cheylan 1988), possibly favoring wide molars able to cope with a broad range of food items.

Strikingly, this parallel evolution occurs following Pmax. The preexistence of widespread variants, easily produced by modulating the same genetic/developmental networks and matching the adaptive requirements, may have facilitated the response to local selective forces, even on a short time scale and without accumulation of important genetic difference. The channeling role of Pmax thus provides a new light on such cases of parallel evolution, apparently amazing but facilitated by the congruence of reduced population size, strong selective pressure, and agreement between the direction of least evolutionary resistance (Pmax) and the selected shape change.

Developmental mechanisms and functional constraints beyond Pmax

Pmax corresponds to a variation ranging from slender, elongated molars and to broad, stocky molars. Advances in developmental biology of tooth morphogenesis provide hints about the mechanisms that may underlie such morphological variation. Concerted changes in the size of all cusps may be mediated by changes in the regulation of genes controlling tooth development (Mustonen *et al.* 2003), a change relatively 'easy' to produce. This explains why corresponding variants are frequent in a population, and why this kind of variation can be easily mobilized by selection and drift to cause pronounced differences among populations even in relatively short time span.

In some cases, the change involved is more localized and the molar elongation seems to involve mostly the anteriormost part of the tooth. This localized variation may be attributed to another developmental process, namely a differential incorporation of a vestigial bud anterior to the molar field and reminiscent of a premolar present in murine ancestors (Peterková *et al.* 2005; Prochazka *et al.* 2010). This component of molar shape variation is very pronounced in the house mouse, leading to the occasional occurrence of an anterior cusplet (Renaud *et al.* 2011). Such a cusplet is rare, but

sometimes present, in some populations of wood mice (Fig. 1). This shows shared developmental potentialities across species however displaying marked differences in their dental pattern, having diverged more than 10 million years ago.

Both developmental mechanisms change the shape of the tooth without perturbing the longitudinal alignment of the cusps. This matches a strong functional requirement during occlusion of the molars. Murine rodents chew along a longitudinal (propalinal) direction. In agreement, the arrangement of the cusps evolved into longitudinal rows that slide into gutters in the occluding molar row (Lazzari *et al.* 2008). Changes related to Pmax, being the concerted spacing of all cusps or a local anterior elongation, occur in agreement with this constraint. Intra-population variants along Pmax, and evolution along this direction, are thus prone to occur because such mice, at any step of their evolution, display functional molar rows despite variation in their overall shape.

Conclusion

This study aimed at considering evolutionary patterns in wood mouse molar shape to the light of the intra-population variation as possible 'line of least resistance to evolution'. It showed the potential of this approach to reconsider striking cases of parallel, pronounced and fast evolution, mostly on islands. Considering the intra-population variance as a hint for underlying genetic/developmental networks, this means that evolution on islands can produce fast response to selection and/or drift by modulating existing networks. In contrast, only reduced differentiation accumulated between ancient mainland phylogenetic lineages, and the shape changes involved were independent of the direction of main intra-population variance. Evolution among the continental lineages was probably constrained by stabilizing selection in this generalist species, and possibly involved small genetic changes not related to the networks producing the main variation, accumulating together with genetic divergence. The questions opened by such approaches challenge further studies, integrating evolutionary, ecological, functional and developmental investigations, that are needed to develop a comprehensive interpretation of the processes underlying morphological diversification in an 'eco-evo-devo' perspective.

Acknowledgements

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Country	Locality		Code	Group	Genet	M/ISL	UM1	Collection
Belgium	Dalhem		DAL	NE	WEUR	M	16	JRM
	Awan		AWAN	NE	WEUR	M	3	JRM
France	Montpellier		MTP		WEUR	M	17	JRM
	Cap Lardier		CAPL	FR-SE	WEUR	M	6	JRM
	Esterel		ESTER	FR-SE	WEUR	M	2	JRM
	La Penne		LA-PE	FR-SE	WEUR	M	4	JRM
	Banyuls		BAN		WEUR	M	10	JRM
	Ménigoute		MEN	FR-W	WEUR	M	2	JRM
	St Brisson		ST-B	NE	WEUR	M	8	JRM
	Ispagnac		ISP		WEUR	M	3	JRM
	Lignières		LIGN	FR-W		M	10	MNHN
	St Méen		ST-ME	FR-W		M	6	MNHN
	Puceul		PUC	FR-W		M	18	MNHN
	Nozay		NOZ	FR-W		M	3	MNHN
	Gardouch		GARD			M	9	CBGP
	Ferel		FER	FR-W		M	2	CBGP
	St Michel en l'Herm		ST-M	FR-W		M	2	CBGP
	Tourch		TOU	FR-W		M	88	CBGP
	Mimizan	Grand Mail	M-GM			M	18	CBGP
		Petit Mail	M-PM			M	10	CBGP
		Leslurgues	M-LES			M	13	CBGP
		Sequedey	M-SEQ			M	22	CBGP
	Lantabat	Feb. 00	LANT			M	17	CBGP
		Nov. 00				M	14	CBGP
		Nov. 01				M	5	CBGP
		Mar. 02				M	5	CBGP
	Noirmoutier		NOIRM		WEUR	ATL-I	5	JRM+CBGP
	Oléron		OLER		WEUR	ATL-I	15	JRM
	Ré		RE		WEUR	ATL-I	7	JRM
	Yeu		YEU		WEUR	ATL-I	1	JRM
	Porquerolles		PORQ		WEUR	HYE	86	JRM
	Port-Cros		PT-CR		WEUR	HYE	12	JRM
	Corsica	Fango	CO-FA		ITB	COR-S	5	JRM
		Bonifacio	CO-BO		ITB	COR-S	3	JRM
Italy	Tarquinia		TARQ	IT-CW	ITB	M	32	JRM
	Grosseto		GROSS	IT-CW	ITB	M	6	JRM
	IT-N	Alpi Apuane	IT-N&E		ITB	M	1	SR
		Gargano	IT-N&E		ITB	M	1	SR
		Mte Vulturo	IT-N&E		ITB	M	1	SR
	Gambarie		GAMB		ITB	M	2	JRM
	Elba		ELBA		ITB	COR-S	1	JRM
	Sardinia		SARD		ITB	COR-S	13	JRM
	Sicily	Ficuzza	FIC	SIC	SIC	SIC	7	JRM
		Grateri	GRAT	SIC	SIC	SIC	8	JRM
		Marettimo	MARET		SIC	SIC	1	JRM
Spain	Fabian		FAB		WEUR	M	4	JRM
	Figueras		FIG		WEUR	M	5	JRM
	Alcoy		ALC	SP-E	WEUR	M	10	JRM
	Posada		POS		WEUR	M	3	JRM
	Nogais		NOG		WEUR	M	3	JRM
	Murcia		MURCIA	SP-E	WEUR	M	15	JRM
	Ibiza		IBIZA		WEUR	IBIZ	9	JRM
Portugal	Murta		MURTA		WEUR	M	3	JRM
Switzerland	Zurich		ZUR	NE	WEUR	M	2	JRM
Germany	Soest		SOEST	NE	WEUR	M	4	JRM
Tunisia	Ain Dram		AIN-D		NAF	M	8	JRM

Table 1. Localities of trapping presented by country, units used in statistical analyses (Code), groups used for calculating evolutionary directions (Group), genetic lineage (Genet), location on mainland or on an island (M/ISL), number of items (UM1) measured, and origin of the collection (JRM: J.R. Michaux, University of Liège, Belgium; CBGP: Centre de Biologie et de Gestion des Populations, Baillarguet, France; MNHN: Museum National d'Histoire Naturelle, Paris, France; SR: S. Renaud, University Lyon 1, France).

Locality	Factor	Size (A0)		Shape (FCs)
		P_{ANOVA}	P_{KW}	P_{MANOVA}
Lantabat	Sex	0.189	0.267	0.2792
	Season	0.822	0.819	0.6305
Mimizan	Sex	0.128	0.099	0.9528
	Sub-locality	**0.001**	**0.003**	0.0603
Tourch	Sex	0.746	0.991	0.1042

Table 2. Test of sexual dimorphism, seasonal and local variations in molar size and shape. Size was estimated by the zeroth harmonic (A0) of the molar outline and differences were tested using analyses of variance (ANOVA) and Kruskal-Wallis (KW) tests. Shape was estimated by the set of Fourier coefficients (FCs) and differences were tested by multivariate analyses of variance (MANOVA). Probabilities of the respective tests are provided. In bold significant probabilities ($P < 0.05$).

	Lantabat	Mimizan	Tourch	Porquerolles	Tarquinia
%variance	51.1	32.0	36.8	29.3	49.2
$R_{bootstrap}$	**_0.982_**	**_0.962_**	**_0.980_**	**_0.909_**	**_0.969_**
Lantabat	-				
Mimizan	**_0.950_**	-			
Tourch	**_0.973_**	**_0.942_**	-		
Porquerolles	**_0.867_**	**_0.872_**	**0.846**	-	
Tarquinia	**_0.952_**	**_0.911_**	**_0.966_**	0.752	-

Table 3. Correlation between Pmax (first eigenvector of the VCV matrix on the FCs) in five well-sampled populations. The first three populations (Lantabat, Mimizan, Tourch) correspond to Western European mainland. Porquerolles documents an insular population belonging to the Western European clade. Tarquinia corresponds to a mainland population of the italo-balkanic lineage. Percentage of variance explained by Pmax is given in the first row. $R_{bootstrap}$, mean correlation between bootstrapped and original Pmax for each population (100 bootstraps). R values are indicated, significant probabilities based on comparison with correlation between random vectors in bold + underlined ($R = 0.860$, $P < 0.0001$) and in bold ($R = 0.770$, $P < 0.001$).

	R$_{bootstrap}$	Pmax					Evolution		
		LANT	MIM	TOU	TARQ	PORQ	V1$_{mainland}$	WEUR→IT	NE→WF
V1$_{mainland}$	**0.772**	**-0.782**	-0.655	**-0.828**	**-0.833**	-0.619	-		
WEUR→IT	<u>0.975</u>	0.520	0.390	0.606	0.622	0.438	<u>**-0.883**</u>	-	
NE→FR-W	<u>0.957</u>	-0.581	-0.503	*-0.683*	-0.607	-0.510	**0.856**	**-0.830**	-
CONT/Latitude	<u>0.976</u>	**-0.802**	*-0.654*	**-0.804**	**-0.802**	*-0.694*	<u>0.938</u>	**-0.794**	*0.767*

Table 4. Pmax and evolutionary directions. Left, correlations between Pmax and evolutionary directions. Right, correlations among directions of evolution. Directions of evolution are: (1) first axis of intergroup variation among mainland localities (V1$_{mainland}$, calculated as the first eigenvector of the VCV matrix on average FCs per groups, 30.6% of the inter-group variance; N = 33). (2) Differentiation between Western European and Italian lineages. (3) Differentiation between Western France (FR-W) and North-Eastern (NE) representatives of the Western European group. (4) Latitudinal trend, estimated by the multivariate regression of FCs against latitude (N=413).

R values are indicated, significant probabilities based on comparison with correlation between random vectors in bold + underlined (P < 0.0001, R = 0. 860), in bold (P < 0.001, R = 0.770) and in italics (P < 0.01, R = 0.651). R$_{bootstrap}$, mean correlation between bootstrapped and original Pmax for each population (100 bootstraps).

		P	R$_{bootstrap}$	Pmax					Allom-intra		V1	Allom-inter
				LANT	MIM	TOU	TARQ	PORQ	MIM	TOU	V1$_{mainland}$	CONT-GM
Allom-intra	AllomMIM	0.013	**0.799**	- 0.462	- 0.551	- 0.500	- 0.495	- 0.320	-			
	AllomTOU	0.001	**0.822**	- 0.485	- 0.484	- 0.471	- 0.384	- 0.573	0.561	-		
Allom-inter	CONT-GM	<0.001	<u>0.901</u>	0.414	0.299	0.448	0.532	0.282	0.304	0.403	**-0.815**	-
	CONT-Tot	<0.001	<u>0.953</u>	0.216	0.113	0.257	0.279	0.204	*0.652*	0.485	*-0.707*	<u>0.901</u>

Table 5. Pmax and allometry. Allometric directions were calculated based on multivariate regressions of FCs vs. size (A0) (P: probability of the regression). Left, comparison between Pmax and allometric directions, being intra-population estimate (Allom-intra), and inter-populations estimates (Allom-inter; estimated on continental group means (CONT-GM) and all continental specimens (CONT-TOT). Rigth, allometric directions are further compared between them, and to the main direction of inter-group variation on the continent (V1$_{mainland}$).

For correlations among directions, R values are indicated, significant probabilities based on comparison with correlation between random vectors in bold + underlined (P < 0.0001, R = 0. 860), in bold (P < 0.001, R = 0.770) and in italics (P < 0.01, R = 0.651). R$_{bootstrap}$, mean correlation between bootstrapped and original Pmax for each population (100 bootstraps).

$R_{bootstrap/original}$	Pmax					Allom-intra	
	LANT	MIM	TOU	TARQ	PORQ	MIM	TOU
FR-SE→PORQ	**<u>0.976</u>**	**<u>0.909</u>**	**0.852**	**<u>0.908</u>**	**<u>0.881</u>**	**<u>0.858</u>**	-0.399	-0.336
IT-CW→SARD	**<u>0.909</u>**	0.169	0.360	0.178	0.145	0.165	-0.559	-0.418
FR-W→OLER	**<u>0.986</u>**	**0.843**	**0.775**	**<u>0.893</u>**	**0.792**	**0.800**	-0.374	-0.562
FR-W→RE	**<u>0.966</u>**	*0.766*	*0.716*	**0.847**	*0.738*	**0.785**	-0.242	-0.438
IT→SIC	**<u>0.921</u>**	**<u>-0.869</u>**	**-0.847**	**<u>-0.886</u>**	**<u>-0.896</u>**	-0.617	0.485	0.262

Table 6. Pmax, insular evolution, and allometry. Evolutionary divergence characterizing insular populations (differences between FCs of insular specimens and mainland relatives) were compared to Pmax of several populations, and to intra-population allometric direction in the two populations where allometry is significant.

For correlations among directions, R values are indicated, significant probabilities based on comparison with correlation between random vectors in bold + underlined (P < 0.0001, R = 0. 860), in bold (P < 0.001, R = 0.770) and in italics (P < 0.01, R = 0.651).

Figure captions

Figure 1. Examples of first upper molars of the wood mouse (*Apodemus sylvaticus*) in mainland (above) and insular (below) populations. Three molars from Tourch document examples of intra-population variation. Latitudinal variation within Western European phylogenetic lineage is exemplified by molars from Dalhem (Belgium), Montpellier (France) and Murcia (Spain). The Italian clade is exemplified by Tarquinia. Examples of islands are presented below: Oléron, off Western France; Porquerolles, off Southern France, Sardinia, off Italy. Right molars are represented, anterior part above, lingual side to the left.

Figure 2. Molar shape differences among European mainland and insular populations of wood mice. (A) Morphospace defined by the first two axes of a PCA on the Fourier coefficient of the molar outline analysis. Group means are represented. Symbol indicates the main biogeographic groups; symbol size proportional to the number of items per group. Main biogeographic groups are: WF, Western France; FR-MED, French Mediterranean area; IBER, Spanish peninsula; NE, North-Eastern Europe; IT, Italian peninsula; NAF, North Africa; SIC, Sicily; W-ISL, Western French islands; MED-ISL, Mediterranean islands. Reconstructed outlines visualize molar shape at the extremes of the multivariate axes. (B) Mean reconstructed outlines for some groups (upper panel) and shape changes (lower panel) including: mean shape differences involved in some insular differentiation are depicted (to the right); shape changes corresponding to Pmax; and shape changes related to latitude (to the left).

Figure 3. Geographical variation in the wood mouse molar morphology. (A) Molar shape variation across Europe. Molar shape is summarized as mean scores along PC1 (see Fig. 2A), represented as grey level. (B) Molar size variation. Molar size is estimated by mean A0 per localities, represented as grey level. Symbol size is proportional to the number of items per groups.

Figure 4. Relationships between Pmax and directions of evolution. All vectors are projected on the total morphospace (Fig. 2A). Vectors pointing in a similar directions (+ and – arbitrary) suggest that molar shape changes share common components. Note that the morphospace was constructed using 14 shape variables; the correlation of the vectors is thus expressed on a 14-dimensional space. All vectors are here scaled to unity (shown using a circle of radius 1): vectors shorter than unity point into a multivariate direction out of the plane. Full arrows: Pmax estimates in different populations. Grey dotted arrows: evolutionary directions. Dark dotted arrows: allometric directions.

Figure 1

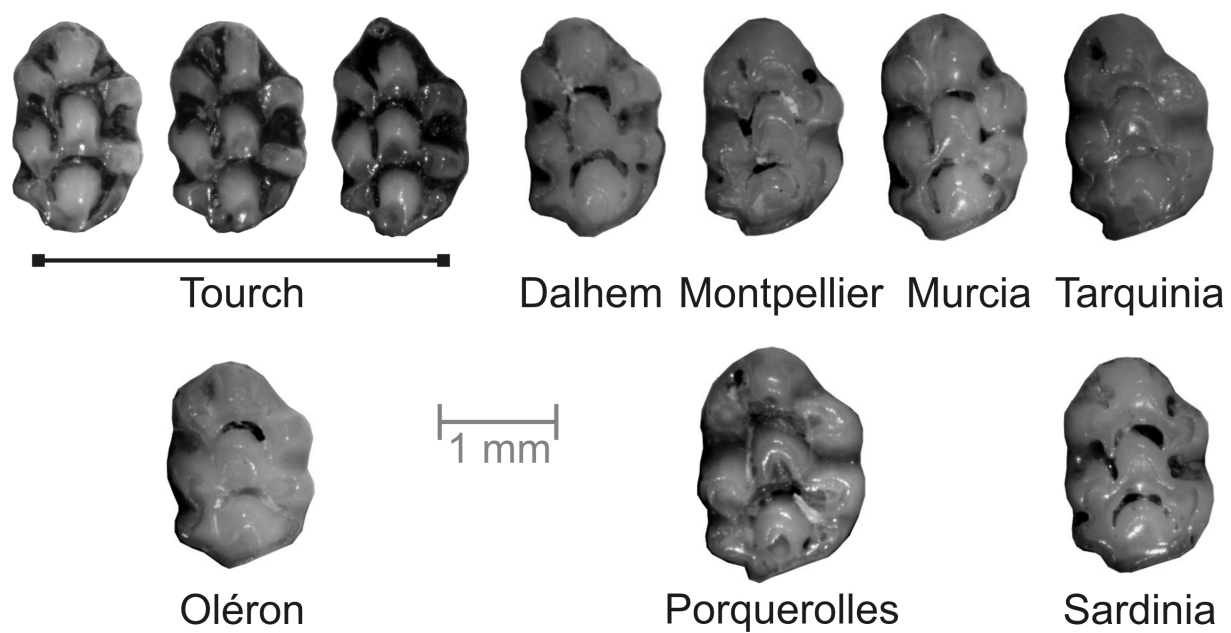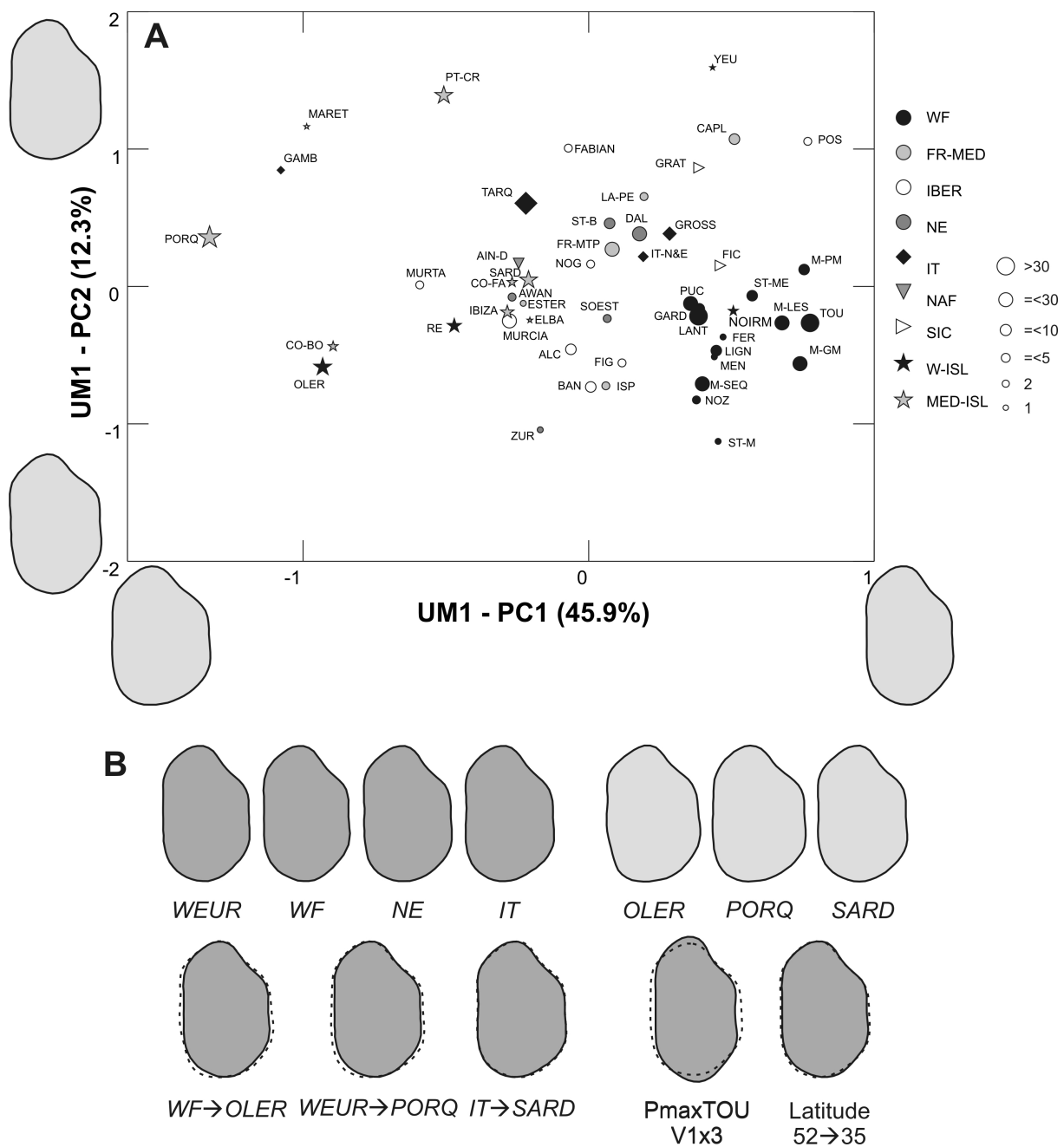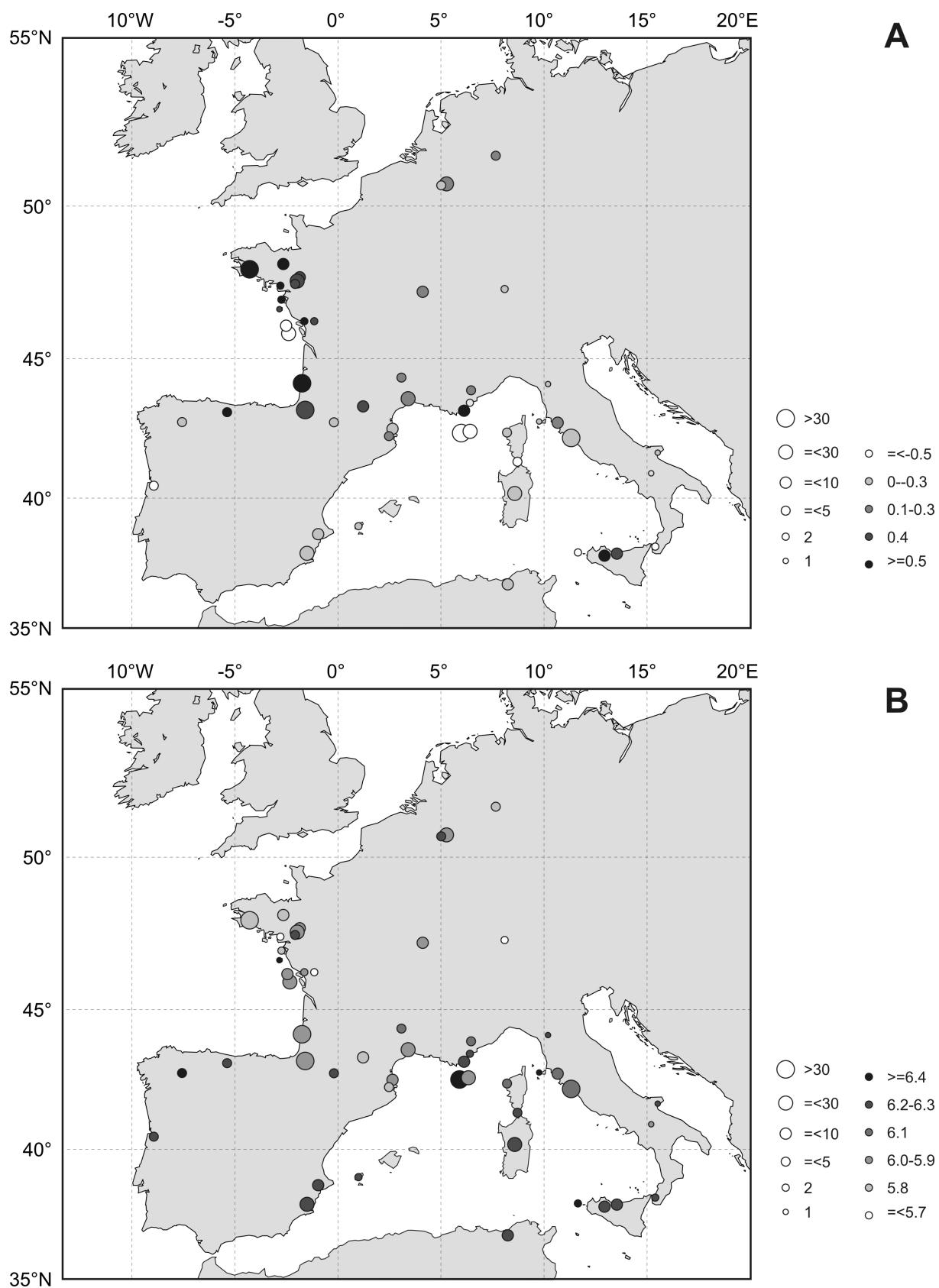Tourch   Dalhem Montpellier Murcia Tarquinia

1 mm

Oléron   Porquerolles   Sardinia

Figure 2

Figure 3

Figure 4