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Phylogeography of the garden dormouse *Eliomys quercinus* in the western Palearctic region

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The garden dormouse, *Eliomys quercinus* (Rodentia, Gliridae), displays a surprisingly high karyotypic diversity, with the number of chromosomes varying between 2N = 48 and 2N = 54. We aimed to assess whether the karyotypic diversity is congruent with the mitochondrial differentiation of the populations; improve our understanding of the taxonomic relationships between garden dormouse populations based on both chromosomal and mitochondrial information; and establish the phylogeographic history of the species and the time of differentiation of mitochondrial lineages of *E. quercinus* and *E. melanurus*. For this purpose we sequenced the mitochondrial cytochrome-*b* (*Cytb*) gene for 62 *E. quercinus* and 4 *E. melanurus* from 27 localities representing all the known chromosomal races of the genus *Eliomys* in the western Palearctic region. Our results 1st showed that populations of *E. quercinus* are separated into 4 evolutionarily significant units partially congruent with the chromosomal races and corresponding to Iberian (2N = 48), Italian (2N = 48 or 50), western European (2N = 48, 49, or 50), and Alpine (2N = 52 or 54) mitochondrial lineages or clades. The existence of hybrid individuals between chromosomal races and the presence of several chromosomal races within each mitochondrial lineage both indicate that gene flow persists between chromosomal races. Second, we estimated that the major mitochondrial lineages differentiated from each other around 4.2 ± *SD* 1 million years ago, thus predating the Quaternary glaciations. Third, *E. quercinus* displayed a higher haplotypic variability in the Mediterranean peninsula than in the northwestern European populations. However, *E. quercinus* did not show a pattern of postglacial recolonization of northwestern Europe from Iberian or Italian populations. Our results also suggest that additional, unexpected refuge regions around the Alps exist for the species. Such information will be useful for deciphering the priorities for the protection of *E. quercinus*, which is listed as "Near Threatened" on the International Union for the Conservation of Nature *Red List of Threatened Species* and is protected by Appendix III of the Bern Convention.

Key words: chromosomal race, *Cytb*, cytochrome-*b* gene, *Eliomys quercinus*, karyotypic diversity

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The garden dormouse, *Eliomys quercinus*, is a rodent from the family Gliridae. The species is listed as "Near Threatened" on the International Union for the Conservation of Nature *Red List of Threatened Species* (International Union for the Conservation of Nature 2011) and is protected by Appendix III of the Bern Convention (The European Community, decision 82/72/EEC, Official Journal L of 10.02.1982) because populations decreased significantly in the last 2–3 decades, particularly in the eastern part of its distribution. Yet, occasionally the species remains considered as a pest in orchards and when individuals hibernate in houses, and its flesh is consumed by humans in some areas (Carpaneto and Cristaldi 1995). The observed decline in population size may be caused by the increase in monoculture agriculture and the use of pesticides, which reduce the amount of food available for dormice, and by competition with the brown rat, *Rattus norvegicus* (Cristaldi and Canipari 1976; Macdonald and Barrett 1993). Population decline may lead to inbreeding, potentially posing a threat to the long-term survival of the species across its distribution (Charlesworth and Willis 2009;

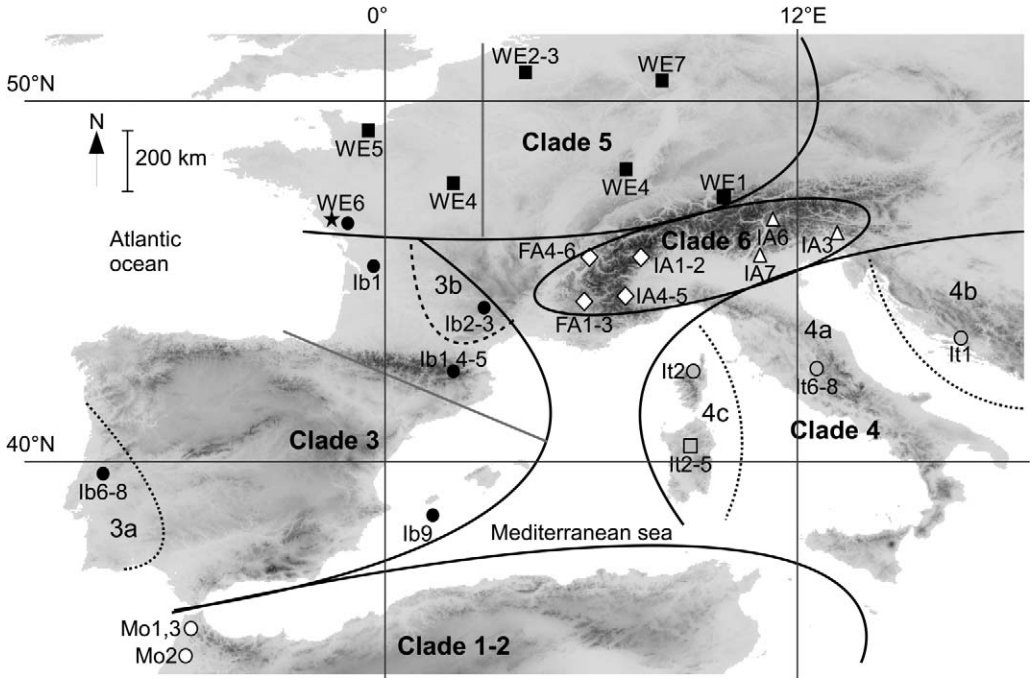

Fig. 1.—Geographic distribution of samples of *Eliomys quercinus* and *E. melanurus*. The number of chromosomes for each karyotype is represented by a symbol: for *E. quercinus* black squares for the 50-chromosome "northern" karyotype, a gray square for 50-chromosome "Sardinian" karyotype, black circles for the 48-chromosome "Iberian" karyotype, gray circles for the 48-chromosome "Italian" karyotype, triangles for the 52-chromosome "Alpine" karyotype, diamonds for the 54-chromosome "Alpine" karyotype, white circles for the 46-chromosome *E. melanurus*, and a star for the hybrid bearing 49 chromosomes (also see Table 1). The bold lines indicate the population subdivisions between clades, the dotted lines the subdivisions between subclades, and the gray lines distinguish southern and northern, and western and eastern, groups of populations in clades 3 and 5 used in Table 2.

Keller and Waller 2002; Saccheri et al. 1998). It thus becomes relevant to determine the population genetic structure of the species in order to identify evolutionarily significant units (Fraser and Bernatchez 2001; Moritz 1994). Determining the biogeography of unique lineages of *E. quercinus* will allow conservation biologists to specify which regions contain evolutionarily distinct groups and their conservation priority.

The species is mainly distributed in the Palearctic region, although several species of the genus *Graphiurus* are found in southern and central Africa. *E. quercinus* is widespread in southwestern Europe, in most of the western Mediterranean islands, and in localized areas from central and northern Europe (Fig. 1). Similar to all Gliridae from temperate regions, *E. quercinus* displays a long period of hibernation and an arboreal lifestyle. The garden dormouse is more terrestrial than the other representatives of its family and is most abundant in rocky zones, orchards, and gardens (Bertolino 2007; Bertolino and Cordero di Montezemolo 2007; Bertolino et al. 2003; Filippucci 1999). Its diet is omnivorous and is composed of insects, fruits, and small vertebrates such as hatchlings or small birds and mammals (Gil-Delgado et al. 2009, 2010).

Information about the population genetic structure of *E. quercinus* is available from karyotypic data, which show a surprisingly high variability across the distribution range of the species (Fig. 1; Table 1 and references therein; Libois et al. 2012). Previous studies have shown evidence of sympatry among divergent chromosomal races at specific locations such as Gran Paradiso National Park in Italy (Cristaldi and Canipari 1976) and evidence of hybrids in Vendée (France) where Iberian 48-chromosome and western European 50-chromosome races come into contact. These findings suggest that hybrids between chromosomal races may be viable and that the karyotypic differentiation does not correlate necessarily with reproductive isolation nor with genetic differentiation observed with other molecular markers (e.g., Madeiran house mouse [*Mus musculus domesticus*—Förster et al. 2009]; but see southern African pygmy mouse [*Mus minutoides*—Veyrunes et al. 2010]). Similar patterns of distinct chromosomal races in the sibling species *E. quercinus* and *E. melanurus* have been found across their distribution ranges (Fig. 1; Table 1).

High karyotypic variability has been observed in other mammals, mainly in rodents and insectivores (e.g., the common shrew [*Sorex araneus*—Polyakov et al. 2011], the Madeiran house mouse [*M. musculus domesticus*—Britton-Davidian et al. 2000; Mitsainas and Giagia-Athanasopoulou 2005], and the Brazilian marsh rat [*Holochilus brasiliensis*—Nachman and Myers 1989]); however, such a large karyotypic diversity is usually typical of higher taxonomic levels (e.g., Cetacea and Pinnipedia [Árnason 1972], European rodents [Mitsainas et al. 2008], and the field mouse genus *Calomys* [Salazar-Bravo et al. 2001]). The chromosomal diversity of the genus *Eliomys* is unusually high compared to other Gliridae, for which the number of chromosomes is almost fixed in each species (for a review of the literature, see Table 1). The

Table 1.—Description of the karyotypic diversity found in *Eliomys quercinus* and closely related Gliridae species.

Species	Karyotype	Karyotypic characteristics	Sampled localities	References
Eliomys quercinus	48 Iberian	Displays a single acrocentric pair of chromosomes	Iberian Peninsula, southern France, and Balearic Islands	Arroyo Nombela et al. 1982; Filippucci et al. 1988a; Guardia and Girela 1980; Libois and Ramalhinho 2005; Libois et al. 2012; Ramalhinho and Libois 2001
	48 Italian	Displays 3 pairs of acrocentric chromosomes	Italian peninsula, Dalmatia (southern Croatia), Romania, St. Petersburg (Russia), and on the islands of Corsica, Sicily, and Lipari	Cristaldi and Canipari 1976; Filippucci et al. 1988a; Godena et al. 1978; Gornung et al. 2010; Graphodatsky and Fokin 1993; Murariu et al. 1985; Orsini 1987; Vujosevic et al. 1993
	49	50 "northern"/48 "Iberian" hybrid	Charente (western France)	Libois et al. 2012
	50 northern	Differs from the 48 "Iberian" race by a single Robertsonian fission	Austria, Belgium, Czech Republic, northern France, Germany, and western Switzerland	Arroyo Nombela et al. 1982; Filippucci et al. 1988a, 1990; Leonard et al. 1970; Libois et al. 2012; Tranier and Petter 1978; Zima et al. 1997
	50 Sardinian	Differs from the 48 "Italian" race by a single Robertsonian fission	Sardinia	Libois and Ramalhinho 2005
	52 Alpine		From Mont Blanc (southeastern France) to the northern side of Tirol (Austria) and in the western part of Friuli (northern Italy) by the southern side of Garde Lake	Cristaldi and Canipari 1976; Filippucci et al. 1988a; Ramalhinho and Libois 2005; Renaud 1938
	54 "Alpine"		From Valais (Switzerland) to the Mediterranean Sea on both the eastern and the western sides of the Alps	Cristaldi and Canipari 1976; Filippucci et al. 1988a, 1990; Libois and Bssaibis, pers. comm., Université de Liège.
Eliomys melanurus	46		Maghreb (Algeria, Morocco, and Tunisia)	Delibes et al. 1980; Filippucci et al. 1988a, 1990; Tranier and Petter 1978
	48		Negev Desert (Israel)	Filippucci et al. 1988a, 1990
Dryomys nitedula	48		From all populations	Filippucci et al. 1985; Mitsainas et al. 2008; Zima et al. 1995
Glis glis	62		From all populations	Mitsainas et al. 2008; Peshev and Delov 1995
Muscardinus avellanarius	46		From almost all populations	Peshev and Delov 1995; Şekeroğlu et al. 2011; Zima et al. 1995
	48		Switzerland	Renaud 1938

unusually high karyotypic diversity of *E. quercinus* thus deserves an explanation of the extent of gene flow existing between races, especially because populations with different numbers of chromosomes can live in sympatry (in the Alps) and because hybrid individuals can be found at the margins of distinct chromosomal races (in Vendée, France).

In addition to chromosomal variation observed within *E. quercinus*, large morphological variation is present across the distribution of this species (Filippucci et al. 1988a; Kryštufek and Kraft 1997). However, this morphological diversity does not correspond to chromosomal races, which has led to uncertainties regarding the taxonomic status of garden dormouse populations (Cristaldi and Canipari 1976; Filippucci et al. 1988a). In the past decades, 5 or more species have been described in the genus *Eliomys* across Europe (Miller 1912, cited in Kryštufek and Kraft 1997). These taxonomic changes can have important consequences on the protection status of garden dormouse populations and thus on the long-term persistence of the garden dormouse karyotypic and morphological diversities. The biogeographic history and the genetic structure of garden dormouse populations thus need to be assessed using independent molecular markers.

In this context, we produced a molecular phylogeography by sequencing the cytochrome-*b* (*Cytb*) gene for 66 individuals of *Eliomys* distributed over almost the entire distributional range of the species. Our aims were to assess the level of congruence between the previously identified chromosomal races and the identified *Cytb* mitochondrial lineages; improve our understanding of the taxonomic relationships between garden dormouse populations based on both chromosomal and mitochondrial information; and establish the phylogeographic history of the species including the existence and the localization of former refuge regions during the Quaternary ice ages, the routes of colonization of some Mediterranean islands, and the time of differentiation of mitochondrial lineages of *E. quercinus* and *E. melanurus*, by comparison of

chromosomal, mitochondrial, and paleontological data with the phylogeographic patterns of species with similar ecological niches. Specifically, we hypothesize that there are at least 2 main glacial refugia on the Iberian and Italian peninsulas, each bearing one of the major chromosomal races (48 or 50 chromosomes [see Table 1; Filippucci et al. 1988a]); and populations on islands originated from recent colonization by human transport. Such information will be useful for deciphering the priorities for the protection of populations of *E. quercinus* in case it becomes needed.

Materials and Methods

Sample collection and sequencing.—A total of 66 individuals of *Eliomys* from 27 localities spread over 10 countries of western Europe and the Mediterranean Basin were analyzed, including 56 individuals for which the karyotype was previously established (Table 1). DNA from *Eliomys* sp. was extracted from approximately 10 mg of tissue conserved in alcohol using a cetyltrimethylammonium bromide protocol following Goüy de Bellocq et al. (2001). Dry DNA pellets were diluted in 30 μl of ultrapure water for polymerase chain reaction amplification.

Respectively, 792 or 718 base pairs (bp) of the *Cytb* gene from the mitochondrial genome were amplified using the primers 1F (5′-CCG CTA TAT ACA CGT AAC GC-3′) and 1R (5′-CTG GTT GCC CAC CGA TTC AGG T-3′), or 2F (5′-GGA-CGC-GGR-ATT-TAC-TAY-GG-3′) and 2R (5′-TTC-AGG-TYA-GGG-TTA-GGA-GGT-C-3′). Amplification reactions were carried out in a volume of 20 μl including 2 μl of 10x reaction buffer + Mg (Roche), 0.8 μl of 25 mM $MgCl_2$ (Roche), 1 μl of 10 μM of each primer, 4 μl of 5 M Betaine (Sigma), 0.16 μl of 5 U/ml Taq DNA polymerase (Roche), and 1 μl of genomic DNA at 50–300 ng/μl. Amplification was performed using an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems) with 40 cycles (45 s at 94°C, 45 s at 49°C, and 120 s at 72°C) after an initial step at 94°C for 4 min and a final extension step at 72°C for 10 min. Amplification of degraded samples was done with a "touch-down" procedure for which the annealing temperature was decreased by 0.5°C per cycle starting at 55°C to 49°C in the first 14 cycles and then maintained at 49°C for the last 21 cycles. Polymerase chain reaction products were purified with Exo-SAP kit (USB) and both strands were sequenced by Macrogen Netherlands on a 23 ABI 3730XLs Applied Biosystems sequencer (Applied Biosystems).

Phylogenetic and network analyses.—Cytochrome-*b* gene sequences were aligned using BioEdit version 7.0.5.3 (Hall 1999) and the edible dormouse (*Glis glis*) and forest dormouse (*Dryomys nitedula*) were used as outgroups. The mean transition to transversion ratio was estimated using the computer program Arlequin 3.1 (Excoffier et al. 2006) and the nucleotide frequencies were provided by MEGA5 (Tamura et al. 2011). The Akaike information criterion and Bayesian information criterion were both used in jModelTest 0.1.1 (Posada 2008) to determine the best-fit substitution model for the garden dormouse phylogenetic reconstructions, which was HKY85+G (Hasegawa et al. 1985) with gamma a = 0.187. Phylogenetic reconstructions were performed by maximum likelihood in PhyML 3.0 (Guindon et al. 2010) and MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The robustness of inferences was assessed by bootstrap resampling (Felsenstein 1985) in PhyML using 10,000 random maximum-likelihood repetitions. Bayesian posterior probabilities were obtained from the 50% majority rules consensus of trees sampled every 100 generations for 2,000,000 generations, after removing trees obtained before chains reached stationarity (burn-in determined following authors' recommendations for 10,000 generations). Networks were constructed using the minimum spanning network method (minspnet in Arlequin 3.1—Excoffier et al. 2006), median-joining network (Network 4.600 [www.fluxus-engineering.com]—Bandelt et al. 1999), and maximum-parsimony network method (TCS 1.21—Clement et al. 2000).

Phylogeographical and genetic structure analyses of Eliomys quercinus.—A "mismatch distribution" of substitutional differences between pairs of haplotypes was calculated within each of the main mitochondrial lineages and compared with the Poisson model using DnaSP version 5.10 (Librado and Rozas 2009). This analysis tests for evidence of a recent range expansion. Population genetic structure and differentiation of *E. quercinus* were determined by analyzing the molecular variance (analysis of molecular variance [AMOVA]; available in Arlequin 3.1 program [Excoffier et al. 2006]). We used this method to estimate how the genetic variability observed between haplotypes is distributed across 3 hierarchical levels of population subdivisions. We defined these population subdivisions based on the phylogenetic structure and geographical distribution of haplotypes among the 4 mitochondrial lineages (clades hereafter) identified by the phylogenetic analyses (see Figs. 1 and 2); among the 23 populations within each mitochondrial lineage (Fig. 1; Table 1; Appendix I); and within populations. Haplotypes were assigned to the different groups independently from the information about chromosomal races to allow the comparison of the results gathered by both approaches.

Nucleotide (π) and haplotype (h) diversities were estimated using DNAsp 5.10 (Librado and Rozas 2009), whereas genetic divergence (GD) among the groups of samples were obtained using a distance analysis (Kimura's 2-parameter distance estimator [K2P—Kimura 1980] in MEGA5.05 [Tamura et al. 2011]). π, h, and mean GD were compared among populations belonging to the same clade (intraclade values) and populations belonging to different clades (interclade values); among western European and Alpine clades versus Iberian and Italian clades to assess whether nucleotide diversity was higher within the potential refuge regions as compared to northern populations; between more northern and more southern populations within the Iberian clade (Formentera and Portugal versus Charente, Lozère, and Pyrenees populations [Fig. 1; Table 2) and between the western and eastern populations of the western European clade to assess whether the postglacial colonization

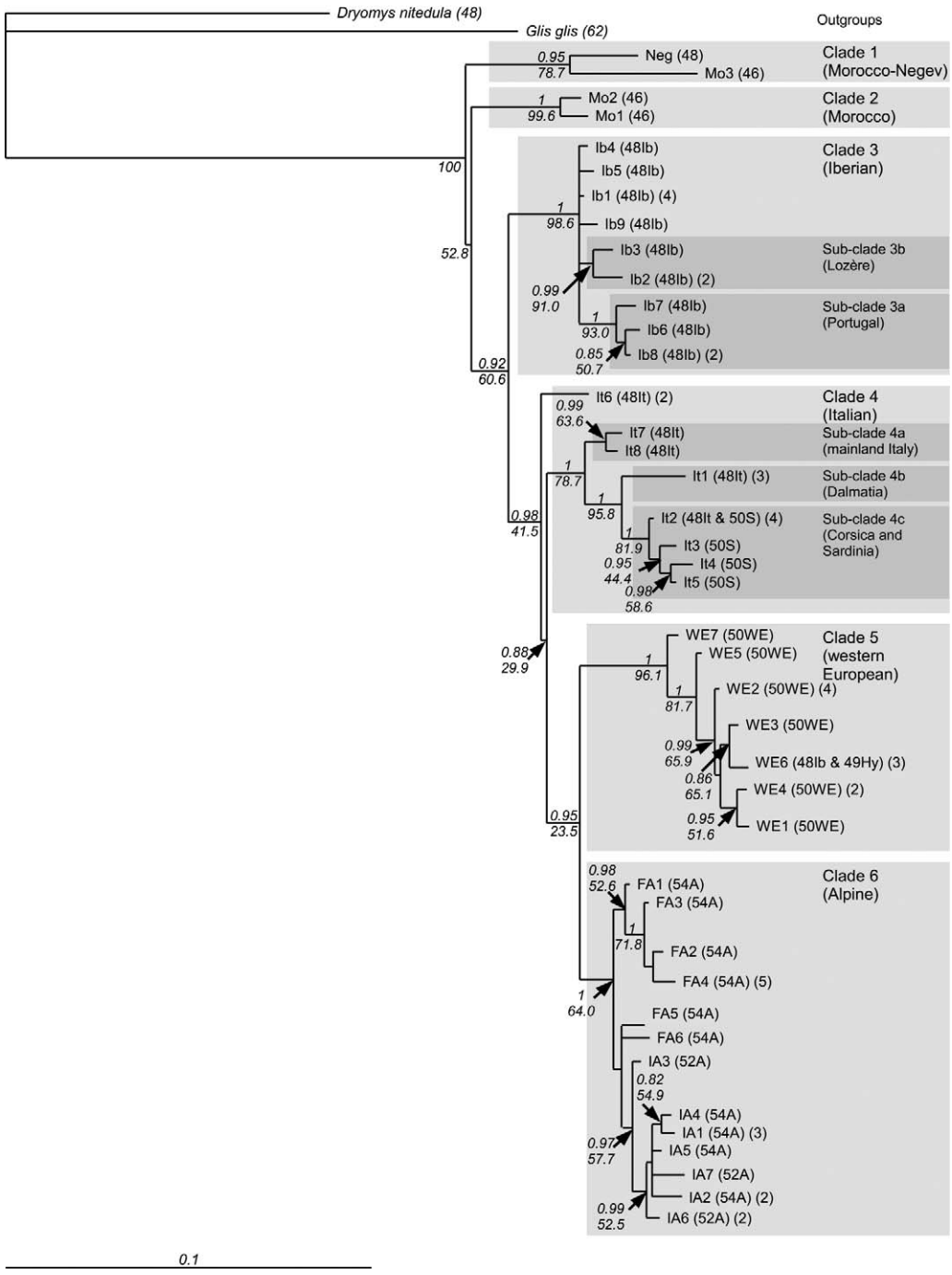

Fig. 2.—Most likely tree of the PhyML reconstruction for the 37 cytochrome-*b* gene haplotypes of *Eliomys quercinus*, 4 haplotypes of *E. melanurus*, and the outgroups *Glis glis* and *Dryomys nitedula*. Information in the 1st pair of brackets indicates the karyotype and the number in the 2nd pair of brackets indicates the number of individuals when more than 1 has the same haplotype (see Table 1 and Fig. 1 for sampling locations). Numbers on branches indicate posterior probabilities in MrBayes analysis (above), when available for the node, and percentage of bootstrap support in the PhyML analysis (below). Note that posterior probabilities under 0.8 and bootstrap values under 50% are not shown.

followed the western European axis (Loiret, Normandy, and Vendée versus Belgium, Haut-Rhin, Rhineland-Palatinate, and Vorarlberg populations [Fig. 1]); and between insular and the most closely related mainland populations (mainland Italy and Dalmatia populations versus Corsica and Sardinia) to assess whether genetic diversity decreased following expectations of the island syndrome (Whittaker and Fernández-Palacios 2007).

Estimation of divergence times between mitochondrial lineages.—Because populations of recent origin may not be at genetic equilibrium, the estimate of the timing of intraspecific divergence must be interpreted cautiously. Therefore, an approximate timing of divergence between the observed mitochondrial lineages was calculated on the basis of the percentage of GD obtained with a distance analysis (K2P

TABLE 2.—Genetic variability observed within the 4 main mitochondrial lineages and groups of populations of *Eliomys quercinus*. Number of haplotypes, K2P genetic divergence (GD), and nucleotide (π) and haplotype (h) diversities in all individuals of *E. quercinus* sampled within a clade (intraclade) and sampled in different clades (interclade), subclades, and groups of populations.

Clade	Subclade or group	Sample size	No. haplotypes	GD (%) ± *SD*	π ± *SD*	h ± *SD*
Alpine	Intraclade	21	13	0.496 ± 0.178	0.005 ± 0.001	0.852 ± 0.003
Iberian	Intraclade	14	9	0.775 ± 0.249	0.008 ± 0.001	0.923 ± 0.060
	Northernmost populations	9	7	0.519 ± 0.162	0.005 ± 0.001	0.867 ± 0.107
	Southernmost populations	5	4	0.666 ± 0.188	0.007 ± 0.002	0.900 ± 0.161
Italian	Intraclade	14	8	1.02 ± 0.301	0.014 ± 0.002	0.890 ± 0.004
	Corsica and Sardinia	7	4	0.380 ± 0.148	0.004 ± 0.001	0.714 ± 0.181
	Mainland Italy and Dalmatia	7	4	1.44 ± 0.342	0.014 ± 0.002	0.810 ± 0.130
Western European	Intraclade	13	7	0.331 ± 0.124	0.003 ± 0.001	0.872 ± 0.067
	Eastern populations	8	5	0.304 ± 0.113	0.003 ± 0.001	0.786 ± 0.151
	Western populations	5	3	0.283 ± 0.135	0.003 ± 0.001	0.700 ± 0.218
Intraclade $\bar{X}$				0.654 ± 0.219	0.013 ± 0.004	0.903 ± 0.038
Interclade $\bar{X}$		62	37	2.50 ± 0.428	0.024 ± 0.001	0.961 ± 0.010

distance—Kimura 1980), and was corrected for ancestral mitochondrial DNA (mtDNA) polymorphism, as proposed by Avise (2000) using the formula:

$$P_{net} = P_{AB} - 0.5(P_A + P_B), \quad (1)$$

where P_{net} is the corrected distance between the isolated lineages A and B, P_{AB} is the mean genetic divergence in pairwise comparisons of individuals A versus B, and P_A and P_B are mean genetic divergence among individuals within these lineages. We calibrated the absolute rate of molecular evolution of *Cytb* gene sequences based on the estimation of divergence time between *E. melanurus* and *E. quercinus* estimated to 7 million years ago (mya) and derived from paleontological and molecular data (Montgelard et al. 2003). Because of unexpected high divergence between the 2 clades of *E. melanurus* (5.58% ± *SD*0.78%), which may reflect the existence of 2 cryptic species, we considered the ancestral genetic diversity of this species as the mean of each intraclade diversity.

RESULTS

Phylogenetic and network relationships of haplotypes.—A total of 37 haplotypes were identified among the 62 *Cytb* gene sequences of *E. quercinus* (EMBL accession numbers HE611090–HE611093 and HE613976–HE614012). Aligned sequences provided 783 bp for analysis, of which 103 sites were variable and 71 were parsimony informative. The mean transition to transversion ratio was 4.38 and the nucleotide frequencies are 29.1%, 26.9%, 13.8%, and 30.3% for A, C, G, and T, respectively. The number of substitutions between haplotypes ranged from 1 to 34. The *Cytb* gene of 3 individuals of *E. melanurus* from Morocco and of 1 *E. melanurus* from Israel also were sequenced, providing 4 additional haplotypes.

We performed a maximum-likelihood reconstruction analysis on the complete haplotype data set of *Eliomys* sp. In addition, the *Cytb* haplotypes from 2 species were used as outgroups, the edible dormouse (*G. glis*; GenBank accession number AJ001562—Reyes et al. 1998) and the forest dormouse (*D. nitedula*; GenBank accession number AJ225116—Bentz and Montgelard 1999). The 4 sequences of *E. melanurus* segregated in 2 well-distinct mitochondrial lineages (i.e., clades) using the maximum-likelihood reconstruction (bootstrap resampling percentage, BT hereafter, and posterior probabilities, PP hereafter; 78.7% and 99.6% BT, 0.95 and 1.00 PP; Fig. 2). One clade grouped the Israeli and Moroccan individuals (clade 1, Morocco–Negev, 78.7% BT, 0.95 PP; Fig. 2), and the other one grouped the 2 other Moroccan individuals (clade 2, Morocco, 99.6% BT, 1.00 PP; Fig. 2), suggesting a strong differentiation among the North African populations.

Four relatively well-supported genetic clades could be distinguished within *E. quercinus* (Figs. 1 and 2): the 1st one included Iberian and southern French populations (clade 3, 98.6% BT, 1.00 PP; hereafter Iberian clade), the 2nd included Italian mainland populations plus populations from Corsica, Sardinia, and Dalmatia (clade 4, 78.7% BT, 1.00 PP; Italian clade), the 3rd covered the northernmost populations of western Europe, namely Austria, Belgium, the northern half of France, and Germany (clade 5, 96.1% BT, 1.00 PP; western European clade), whereas the Alpine populations formed the 4th clade (clade 6, 64.0% BT, 1.00 PP; Alpine clade). One additional haplotype from mainland Italy was next to the Italian clade. There was no clear phylogenetic relationship among the 4 *E. quercinus* clades but the Iberian clade seemed to be weakly differentiated from all other clades (60.6% BT, 0.92 PP, Fig. 2) and the Alpine and western European clades were more closely related to each other than to the Italian clade (23.5% BT, 0.95 PP; Fig. 2).

The Iberian clade was further differentiated into 2 distinct allopatric subclades in Portugal (subclade 3a, 93.0% BT, 1.00 PP) and Lozère (subclade 3b, 77.3% BT, 0.99 PP); the remaining ones could be grouped into a group called "Pyrenees." The Italian clade displayed 3 allopatric subclades: mainland Italy (subclade 4a, 63.6% BT, 0.99 PP), Dalmatia with a single haplotype (subclade 4b, 95.8% BT, 1.00 PP), and Corsica and Sardinia (subclade 4c, 81.9% BT, 1.00 PP). The western European clade displayed 2 well-diverged haplotypes, namely from Rhineland-Palatinate and Normandy (81.7% and 65.9% BT, 1.00 and 0.99 PP, respectively). Finally, there was

low support in the Alpine clade for a segregation between haplotypes that roughly coincided with the geographical barrier formed by the Alps, with one group including all Italian Alps populations (BT 57.7%, 0.97 PP) and the other grouping all but 2 haplotypes from the French Alps populations (52.6.0% BT, 0.98 PP; Fig. 2).

The minimum spanning network using the 37 haplotypes of *E. quercinus* and 4 haplotypes of *E. melanurus* showed that genetic differentiation among groups of haplotypes was large (5–14 mutational steps in *E. quercinus*, i.e., 0.008–0.022 mutational steps by base pair; and 7–31 in *E. melanurus*, i.e., 0.009–0.040 mutational steps by base pair; Fig. 3). The network showed a general congruence with the phylogenetic reconstruction, because the same 6 clades and subclades of *Eliomys* sp., as defined above, could be found. Yet the Italian clade displayed a strong differentiation among subclades (10 mutational steps); whereas in contrast, Italian and Alpine clades showed the lowest differentiation (5 mutational steps).

The median-joining and TCS networks mostly corroborated the topology observed in the minimum spanning network (data not shown). Both reconstructions showed a high differentiation between clades and supported the same topology between subclades, except for the median-joining network that linked the clades of *E. melanurus* to *E. quercinus* via the Iberian clade. The median-joining network also differed from the minimum spanning network by including the haplotypes from Lozère with the haplotypes from Portugal or with the haplotypes from the Pyrenees Mountains, and grouping all haplotypes from mainland Italy together.

Phylogeographical and population genetic structures.—The AMOVA showed that the majority of the mtDNA variation (77%) was distributed among clades, whereas a low percentage of this variation (15%) was observed among populations within the main lineages (data not shown). Similarly, the comparison of nucleotide, haplotype, and genetic divergence values within versus between clades revealed that most of the nucleotide differences distinguish the different clades (Table 2). Thus, a high degree of genetic divergence differentiated the 4 clades (Fig. 2).

A signature of population growth—a bell-shaped distribution (Fig. 4a)—was present in the distribution of the substitutional differences of the Alpine clade, as would be expected for populations expanding after the last ice age from a relatively small number of founder individuals (Luikart et al. 2001). The same distribution was observed for the western European clade and to a lesser extent for the Iberian clade (Fig. 4b). When removing the sequences from Portugal and Lozère from the Iberian clade, the bell-shaped distribution was improved (data not shown). Conversely, the mismatch distribution for the Italian clade did not show a bell-shaped distribution and showed rather a multimodal distribution (data not shown), which suggests that the populations have remained stable with large, long-term effective population size.

The Italian clade displayed a higher mean GD and π than other clades (Table 2; Fig. 4). The Alpine and the western European clades showed comparatively low levels of GD and π as did the Corsica and Sardinia subclades. This may reflect genetic bottlenecks or recent population expansion from a small number of founder individuals in Alpine and western European clades and Corsica and Sardinia subclades (Avise 2000). The Iberian clade showed intermediate values of GD and π. The π, h, and GD values for the western, potential refuge regions, and for the more eastern populations within the western European clade, or for the southern and northern populations of the Iberian clade, were similar (Table 2).

Estimation of divergence time between mitochondrial lineages.—Applying the correction method of ancestral polymorphism, we found an average genetic divergence of 0.46% $\pm$ *SD*0.05% K2P genetic divergence per million years, which is in the lowest range of values recorded for mammals (Avise et al. 1998). The divergence time between the Iberian clade of *E. quercinus* and the others was estimated at 4.5 $\pm$ *SD*0.83 million years ago (mya); between the Alpine, Italian, and western European clades at 4.2 $\pm$ 1 mya; and between the 2 clades of *E. melanurus* at 5.6 $\pm$ 3.0 mya. The divergence of the mainland Italian, the Corsica and Sardinia, and the Dalmatia subclades was estimated at 4.0 $\pm$ 1 mya, whereas the Portugal subclade diverged from other Iberian populations at 2.0 $\pm$ 0.71 mya and the Lozère subclade at 0.89 $\pm$ 0.14 mya.

Discussion

Relationships between mitochondrial lineages and chromosomal races.—The phylogenetic and network reconstructions revealed the existence of 4 main mitochondrial lineages (i.e., clades) partially reflecting the differentiation in chromosomal races: the Iberian clade with the "Iberian" 48-chromosome karyotype (Table 1); the Italian clade with the "Italian" 48-chromosome karyotype or "Sardinian" 50-chromosome karyotype in Sardinia; the western European clade including all northernmost western European populations with "Iberian" 48-chromosome karyotype, 49-chromosome hybrid karyotype, or "northern" 50-chromosome karyotype; and the Alpine clade with 52- or 54-chromosome karyotypes (Table 1; Figs. 2 and 3). The genetic divergence between these 4 lineages was high, varying from 2.2% to 3.9% of K2P genetic divergence (Kimura 1980). The AMOVA clearly indicated that the majority ($\sim$77%) of haplotype diversity was distributed among these clades (data not shown). The various intraspecific haplogroups of *E. quercinus* diverged between 5.6 and 0.88 mya. Our results show that the genetic differentiation of the mitochondrial lineages of *E. quercinus*, and corresponding chromosome races, predates the Quaternary glaciations. Several subclades in the Iberian clade diverged more recently, revealing that the differentiation process went on during the Quaternary glaciations. Iberian and western European clades are genetically distant, indicating that they did not diverge recently from each other. A single Robertsonian fission likely took place in the western European lineage long before the postglacial northward recolonization from Iberia toward

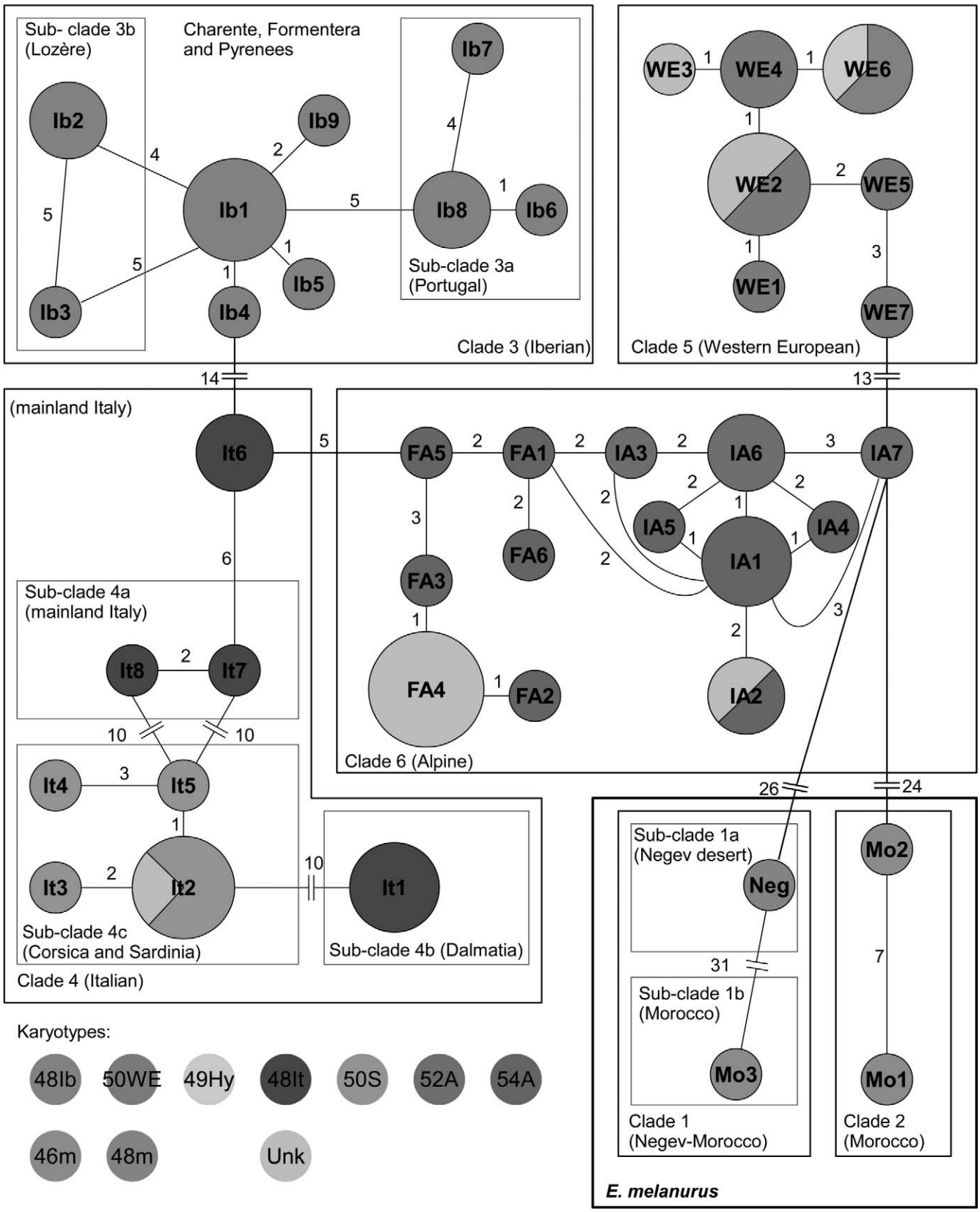

Fig. 3.—Minimum-spanning network using the 41 haplotypes of the cytochrome-*b* gene for *Eliomys quercinus* and *E. melanurus*. The codes used for the haplotypes are the same as in Table 1 and Fig. 1. Numbers correspond to the mutational steps observed between haplotypes, and the size of the circle is proportional to the numbers of haplotypes represented (smallest circle, $n = 1$; largest circle, $n = 5$). Clades and subclades are indicated following the results obtained for the phylogenetic analyses. The karyotype of each haplotype is represented by a color: 48It: "48 Italian," 50S: "50 Sardinian," 48Ib: "48 Iberian," 50WE: "50 western European," 52A: "52 Alpine," 54A: "54 Alpine,"; 46m: "46 *E. melanurus*," 48m: "48 *E. melanurus*," and Unk: "unknown."

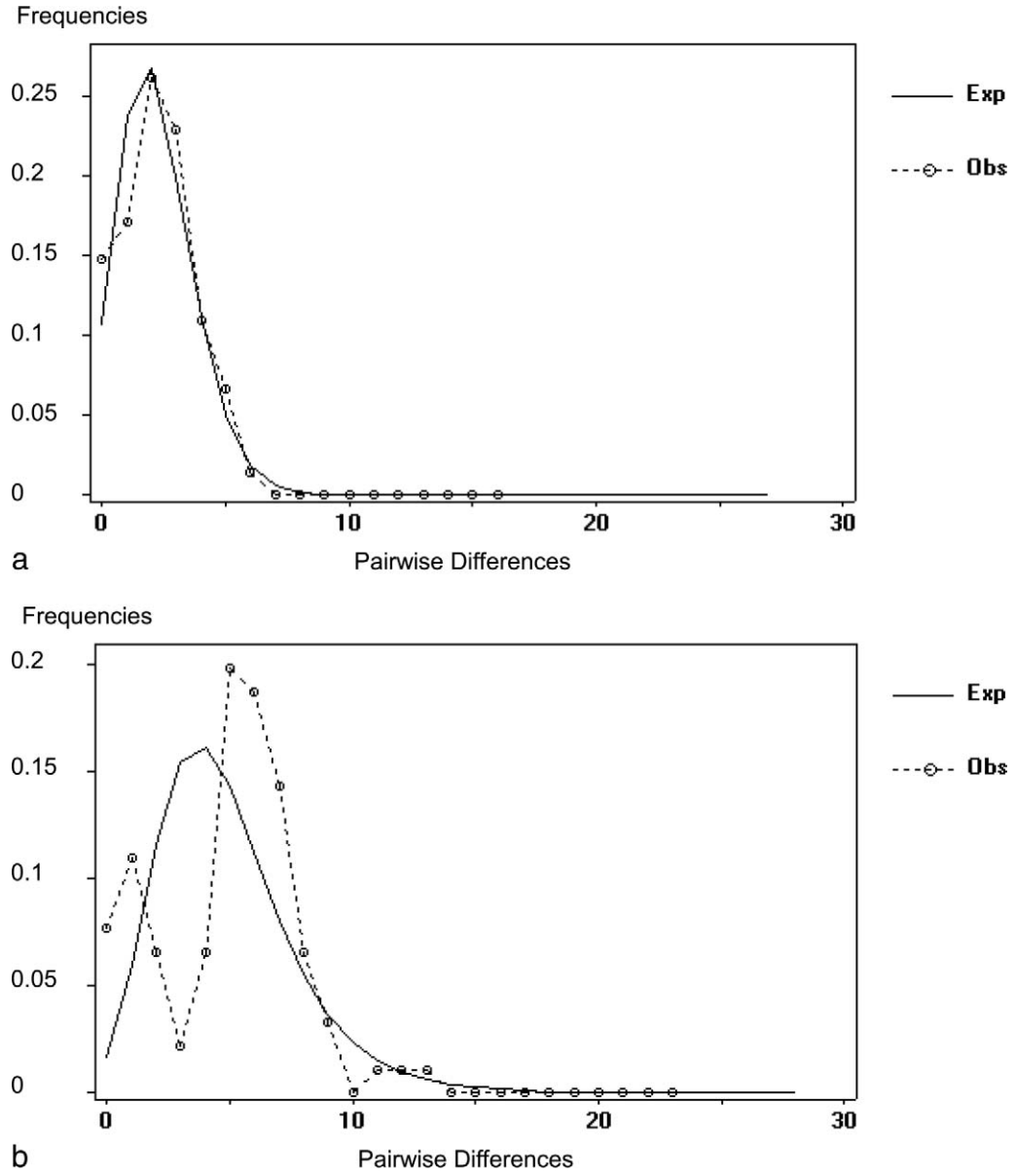

Fig. 4.—Mismatch distribution for *Eliomys quercinus* based on cytochrome-*b* gene sequences for the Alpine and Iberian clades. The expected frequencies (solid line), based on a population growth decline model, using the DNAsp version 5.10 program (Librado and Rozas 2009), are compared to experimental frequencies (dotted line) obtained in the a) Alpine and b) Iberian clades.

western Europe, as proposed by Arroyo Nombela et al. (1982) and Filippucci et al. (1988b).

Incongruities between the chromosomal races and phylogenetic structure occurred in the Alpine clade in which individuals with both 52 and 54 chromosomes were found. In the other clades, 5 haplotypes displayed a chromosomal number differing from that usually found (Fig. 1). The chromosomal variations within each *Cytb* clade can be explained by Robertsonian fissions and other simple chromosome rearrangements; a 48-chromosome karyotype is considered the ancestral karyotype because of its prevalence in the genus *Eliomys* and in the related genus *Dryomys* (Filippucci et al. 1988a). Each incongruity could be explained by the following scenario. The complicated pattern of karypotype–haplotype in Sardinia occurred when a Robertsonian fission took place in an isolated continental population of the "Italian" 48-chromosome clade, which produced the 50-chromosome pattern present in Sardinia (Ramalhinho and Libois 2005). The diversity of karyotypes in the western European clade occurred when a hybridization took place between a western European mother ($2N = 50$) and an Iberian father ($2N = 48$), producing the 49-chromosome hybrid. Such a female (49-chromosome hybrid) was then backcrossed with a 48-chromosome Iberian male to produce an offspring with 48 chromosomes and the western European mtDNA (Table 1; Figs. 1 and 2). Examination of these data suggests that chromosomal hybrids can persist in nature and may thus not suffer large fitness reduction. Further evidence for gene flow among chromosomal

races can be found in northern Africa. Two different chromosome races (46 in Morocco and 48 in Israel; Table 1) belong to the same mitochondrial lineage (clade 1), showing again that gene flow persists between distinct chromosome races.

Demographic history and postglacial recolonization routes of E. quercinus in Europe.—Iberian, western European, and Alpine clades likely underwent a recent population expansion from a small number of individuals after a genetic bottleneck, as suggested by the measures of genetic diversity and the mismatch distribution analysis (Table 2; Fig. 4). The presence of 2 well-differentiated subclades in Portugal and southwestern France, and the absence of a north–south diversity gradient, suggest the existence of allopatric refuges in these regions for the species (Table 2). The paleontological records of *E. quercinus* are numerous in these areas and are recorded from the late Pliocene to the Holocene, including the last glacial age. Other species used these glacial refuges from 3.4 mya to present (Bicho et al. 2003; Kowalski 2001; López Antoñanzas and Cuenca Bescós 2002; López-García et al. 2010; Marks et al. 2002; Povoas et al. 1992; Sesé and Villa 2008; Villa et al. 2010).

The recolonization of western Europe likely did not follow the traditional recolonization routes reviewed by Hewitt (1999), because we could not find a gradient of genetic diversity in either the western European or Iberian clades (Table 2). The western European clade may have rather expanded from a cryptic refuge in continental Europe, as shown in other rodent species (Deffontaine et al. 2005). Such a refuge could be located at the foot of the northwestern Alps where remains of *E. quercinus* have been recorded back to 0.12 mya (Eemian: Côte d'Or, Ardèche) including during the last glacial age (lower Vistulian: Côte d'Or, Jura, Yonne—Chaline et al. 1995; Kowalski 2001). Furthermore, the species is known to be tolerant of cold climates because it is found in the Alps and Pyrenees up to 2,300 m in elevation and in southern Finland (R. Libois, University of Liège, pers. comm.).

In contrast, the higher genetic diversity observed in the Italian clade is likely the result of the long-term survival of populations with relatively high and stable numbers of individuals (Table 2). The presence of differentiated subclades in Italy reveals that the populations differentiated in allopatric refuges, as observed for other species such as the common squirrel (*Sciurus vulgaris*), which shows a distinct lineage in Calabria (Grill et al. 2009), and the wood mouse (*Apodemus sylvaticus*—Avise 2000; Michaux et al. 1998; Nieberding et al. 2005). The paleontological records of the garden dormouse are limited to 0.19 mya to present in the Italian peninsula (Ronchitelli et al. 2011), yet evidence of *Eliomys* sp. has been recorded in the early Pleistocene but has not been clearly attributed to *E. quercinus* (Kotsakis 2003).

Similarly to *E. quercinus*, highly divergent lineages can be found in the edible dormouse (*G. glis*) in Italian populations (Hürner et al. 2010). The Italian lineage of *G. glis* expanded throughout the whole distribution range of *G. glis*, indicating that the Italian peninsula was the main glacial refuge area of *G. glis*. The Alps may thus have acted as a stronger barrier to dispersal in *E. quercinus*, because the Italian lineage appears to remain limited within the Italian peninsula and around the Adriatic Sea. Analysis of samples from Romania and Russia with a known karyotype of 48 chromosomes is needed to provide firm conclusions (Graphodatsky and Fokin 1993; Murariu et al. 1985).

We identified the geographic origin of the island populations based on the phylogenetic proximity of insular haplotypes to continental haplotypes (Fig. 1). Moreover, the karyotype of insular individuals was either identical to those of the continental population they likely originated from, or it differed by a single Robertsonian fission. Based on these data Formentera was colonized by Spain (as suggested by Ramalhinho and Libois [2001]), and Corsica and Sardinia populations originated from Italy or Dalmatia via the Italian peninsula (Table 1; Figs. 2 and 3). The earliest records of *E. quercinus* in Sardinia (6,000 years ago—Vigne and Alcover 1985) and in the Balearic Islands were recent (Majorca: 6,000 years ago, and Menorca: 3,300–3,400 years ago [Vigne and Alcover 1985]) and coincided with the arrival of humans in these islands (Alcover et al. 1999; Traveset et al. 2009). Thus, this colonization was the result of passive or active transportation by humans (Dobson 1998; Ramalhinho and Libois 2001). The phylogenetic differentiation of the Sardinian population was more ancient, and reveals that the island population diverged in the mainland before the colonization of the island.

Taxonomic consideration of Eliomys populations.—The time of divergence for lineages of *E. quercinus* was estimated to have occurred around 4.2 mya. In contrast, the closely related species *G. glis* contained lineages that diverged much more recently, between 0.46 and 0.9 mya (Hürner et al. 2010). The contrasting pattern of divergence mirrors measures of genetic diversity obtained for both species, which are higher in *E. quercinus* than in *G. glis* despite the larger sample size for the latter species (62 versus 130 individuals, respectively). The mitochondrial lineages found in *E. quercinus* also are differentiated by a larger amount of genetic divergence (2.2–3.9% K2P distance for *E. quercinus*, and 0.5–1.5% for *G. glis*—Hürner et al. 2010). Finally, a significant amount of genetic variability is distributed within, rather than among, clades in *E. quercinus* (more than 20% of genetic variance is present within clades in the AMOVA) but not in *G. glis* (less than 5%—Hürner et al. 2010). These results are consistent with the more ancient origin of lineages of *E. quercinus* compared to lineages of *G. glis*. However, the maintenance of gene flow between chromosomal races of *E. quercinus*, and the monophyly of the genetic lineages, leads us to propose that populations of *E. quercinus* are part of a single species, as proposed by Filippucci et al. (1988b).

Recently, *E. quercinus* and *E. melanurus* were clearly established as distinct species on the basis of the sequencing of 1 mitochondrial and 3 nuclear genes, and their time of differentiation was dated back to 7.0 ± 0.9 mya (Montgelard et al. 2003; but see Filippucci et al. [1988b] for the estimation of a

more recent divergence event based on an allozyme study), the expected amount of time that allows divergence between mammal species of the same genus (Avise et al. 1998). However, the taxonomic position of the Maghrebi populations of the garden dormouse remains unclear because only 1 individual of the so-called *E. melanurus* was sampled in Israel previously (Montgelard et al. 2003). The Maghrebi populations are thus currently considered as part of *E. melanurus* (Filippucci et al. 1988b) or as part of *E. quercinus* (Kryštufek and Kraft 1997), whereas some propose it is a 3rd species, *E. munbyanus* (Holden 2005).

The comparison of the phylogeographic structure of samples of *E. quercinus* and *E. melanurus* and the information about their chromosomal races provides some insight about the taxonomic status of both species' populations. *E. quercinus* displays a *Cytb* genetic diversity of 2.5% ± 0.43% of K2P genetic divergence (Table 2), which is in the range of values of what is usually found in conspecific phylogroups (Avise et al. 1998).

Sampled individuals of *E. melanurus* are included in 2 distinct clades, 1 of which (clade 2) is phylogenetically closer to lineages of *E. quercinus* than to the other lineage of *E. melanurus* (clade 1; Fig. 1), suggesting the existence of 1 or 2 distinct valid species within so-called *E. melanurus*. The large genetic differentiation, and related divergence time, found between the 2 clades of *E. melanurus* suggest that the 2 clades diverged in allopatry in different areas of the southern Mediterranean Basin, probably following the aridification of North Africa during the Quaternary (Filippucci et al. 1990). Yet, we need additional samples to confirm the distinctiveness of the 2 clades of *E. melanurus*. Interestingly, individuals bearing the same karyotype (46 chromosomes) but distinct mitochondrial lineages (Mo1 and Mo3 haplotypes in clades 2 and 1, respectively) coexist in Morocco and were captured at the same trapping station (Cape Spartel [Fig. 1; Table 1]), suggesting that gene flow is maintained between divergent mitochondrial lineages.

Conversely, 2 different chromosome races (46 in Morocco and 48 in Israel [Table 1]) belong to the same mitochondrial lineage (clade 1), suggesting that gene flow also persisted after the segregation into distinct chromosome races and may still persist at present. The 46-chromosome Maghrebi individual in clade 1 is indeed the result of hybridization between the Maghrebi populations with 46 chromosomes and individuals with 48 chromosomes that would have differentiated in allopatry. Thus, the discrepancies between the mitochondrial data and the chromosomal races do not support the existence of several species within *E. melanurus*, as described by Filippucci et al. (1988b).

The divergence time between *E. quercinus* and *E. melanurus* was estimated at 7.0 mya (Montgelard et al. 2003), and paleontological data attest to the presence of "modern" *Eliomys* sp. in the Iberian Peninsula and in North Africa since the late Miocene (García-Alix et al. 2008). One can imagine that *Eliomys* diverged into at least 2 species following the Messinian Crisis when contacts between Europe and Africa allowed the garden dormouse to colonize North Africa, as shown for other terrestrial vertebrates (Agusti et al. 2006; Dobson 1998).

It appears that chromosomal divergence in *E. quercinus* did not lead to complete cessation of gene flow between mitochondrial lineages because hybrid individuals can be found (e.g., individuals with 49 chromosomes [Table 1]) and individuals with distinct chromosome races can be found within a mitochondrial lineage. We thus suggest that all European garden dormice belong to the species *E. quercinus*, which is formed by at least 4 lineages that can be considered as independent evolutionarily significant units (Fraser and Bernatchez 2001; Moritz 1994). These evolutionarily significant units can be largely identified by their karyotype or by their mitochondrial sequence, but not by morphological traits, which may reflect local adaptations of populations (Filippucci et al. 1988a). Conservation efforts must be focused on each of these evolutionarily significant units in order to conserve intraspecific genetic and chromosomal diversities. Moreover, contact zones between chromosomal races, for instance Charente and the Alps, could be additional targets for conservation efforts, because these regions include both distinct chromosomal races and potentially hybrids between these races.

The phylogeographic pattern is in agreement with paleontological data and indicates an expansion of the distributional range of the species from glacial refuge zones, including the Iberian Peninsula and southern France, the Italian peninsula, and additional, unexpected refuges possibly located in the (north) western part of the Alps, and at the foot of the Alps. Although the differentiations of the clades are ancient, suggesting that these refuges were active long before the start of the Quaternary ice ages, genetic differentiation continued during this period. To improve the estimation of the genetic diversity and recolonization patterns in Europe for the species, additional sampling is needed in mainland Italy, Finland, Poland, Romania, Russia, Slovakia, and in some potential hybridization areas such as central and western France. Moreover, investigations based on nuclear genes may help to clarify these findings and investigations at the population level could provide information on dispersal abilities of the species and inform conservation efforts.

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Literature Cited

Agustí, J., M. Garcés, and W. Krijgsman. 2006. Evidence for African–Iberian exchanges during the Messinian in the Spanish mammalian record. Palaeogeography, Palaeoclimatology, Palaeoecology 238:5–14.

Alcover, J. A., B. Segui, and P. Bover. 1999. Extinctions and local disappearances of vertebrates in the western Mediterranean islands. Pp. 165–188 in Extinctions in near time: causes, contexts, and consequences (R. D. E. MacPhee and H. D. Sues, eds.). Kluwer Academic/Plenum Publishers, New York, New York.

Árnason, Ú. 1972. The role of chromosomal rearrangement in mammalian speciation with special reference to Cetacea and Pinnipedia. Hereditas 70:113–118.

Arroyo Nombela, J., C. Rodriguez Murcia, M. Delibes, and F. Hiraldo. 1982. Comparative karyotype studies between Spanish and French populations of *Eliomys quercinus* L. Genetica 59:161–166.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, Massachusetts.

Avise, J. C., D. Walker, and G. C. Johns. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proceedings of the Royal Society, B. Biological Sciences 265:1707–1712.

Bandelt, H. J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37–48.

Bentz, S., and C. Montgelard. 1999. Systematic position of the African dormouse *Graphiurus* (Rodentia, Gliridae) assessed from cytochrome *b* and 12S rRNA mitochondrial genes. Journal of Mammalian Evolution 6:67–83.

Bertolino, S. 2007. Microhabitat use by garden dormice during nocturnal activity. Journal of Zoology (London) 272:176–182.

Bertolino, S., and N. Cordero di Montezemolo. 2007. Garden dormouse (*Eliomys quercinus*) nest site selection in an alpine habitat. Ethology Ecology & Evolution 19:51–60.

Bertolino, S., N. Cordero, and I. Currado. 2003. Home ranges and habitat use of the garden dormouse (*Eliomys quercinus*) in a mountain habitat in summer. Acta Zoologica Academiae Scientiarum Hungaricae 49:11–18.

Bicho, N. F., J. Haws, B. Hockett, A. Markova, and W. Belcher. 2003. Paleoecologia e ocupação humana da Lapa do Picareiro: resultados preliminares. Revista Portuguesa de Arqueologia 6:49–81.

Britton-Davidian, J., et al. 2000. Environmental genetics: rapid chromosomal evolution in island mice. Nature 403:158.

Carpaneto, G., and M. Cristaldi. 1995. Dormice and man: a review of past and present relations. Hystrix—The Italian Journal of Mammalogy 6:303–330.

Chaline, J., P. Brunet-Lecomte, and M. Campy. 1995. The last glacial/interglacial record of rodent remains from the Gigny karst sequence in the French Jura used for palaeoclimatic and palaeoecological reconstructions. Palaeogeography, Palaeoclimatology, Palaeoecology 117:229–252.

Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. Nature Reviews Genetics 10:783–796.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1659.

Cristaldi, M., and R. Canipari. 1976. A propos de la caryologie du lérot (*Eliomys quercinus*). Mammalia 40:475–488.

Deffontaine, V., et al. 2005. Beyond the Mediterranean peninsulas: evidence of central European glacial refugia for a temperate forest mammal species, the bank vole (*Clethrionomys glareolus*). Molecular Ecology 14:1727–1739.

Delibes, M., F. Hiraldo, J. Arroyo Nombela, and C. Rodriguez-Mukcia. 1980. Disagreement between morphotypes and karyotypes in *Eliomys* (Rodentia, Gliridae): the chromosomes of the central Morocco garden dormouse. Säugetierkundliche Mitteilungen 28:289–292.

Dobson, M. 1998. Mammal distributions in the western Mediterranean: the role of human intervention. Mammal Reviews 28:77–88.

Excoffier, L., G. Laval, and S. Schneider. 2006. Arlequin ver. 3.1. An integrated software package for population genetics data analysis. Computational and Molecular Population Genetics Lab (CMPG), Institute of Zoology, University of Bern, Bern, Switzerland.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Filippucci, M. 1999. *Eliomys quercinus* (Linnaeus, 1766). Pp. 298–299 in The atlas of European mammals (A. J. Mitchell-Jones, et al., eds.). Academic Press, London, United Kingdom.

Filippucci, M. G., F. Catzeflis, and E. Capanna. 1990. Evolutionary genetics and systematics of the garden dormouse, *Eliomys* Wagner, 1840 (Gliridae, Mammalia): 3. Further karyological data. Italian Journal of Zoology 57:149–152.

Filippucci, M., M. Civitelli, and E. Capanna. 1985. Le caryotype du lérotin, *Dryomys nitedula* (Pallas) (Rodentia, Gliridae). Mammalia 49:365–368.

Filippucci, M. G., M. V. Civitelli, and E. Capanna. 1988a. Evolutionary genetics and systematics of the garden dormouse, *Eliomys* Wagner, 1840. I: karyotype divergence. Bollettino di Zoologia 55:35–45.

Filippucci, M. G., E. Rodino, E. Nevo, and E. Capanna. 1988b. Evolutionary genetics and systematics of the garden dormouse, *Eliomys* Wagner, 1840. II: allozyme diversity and differentiation of chromosomal races. Bollettino di Zoologia 55:47–54.

Förster, D., et al. 2009. Molecular insights into the colonization and chromosomal diversification of Madeiran house mice. Molecular Ecology 18:4477–4494.

Fraser, D. J., and L. Bernatchez. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology 10:2741–2752.

García-Alix, A., R. Minwer-Barakat, E. Martín-Suárez, and M. Freudenthal. 2008. Cricetidae and Gliridae (Rodentia, Mammalia) from the Miocene and Pliocene of southern Spain. Scripta Geologica 136:1–38.

Gil-Delgado, J. A., O. Mira, A. Viñals, J. Gómez, N. Banyuls, and C. Vives-Ferrándiz. 2010. Diet of the garden dormouse (*Eliomys quercinus* Linnaeus 1766) in orange groves: seasonal variation and use of available resources. Mammalia 74:147–151.

Gil-Delgado, J. A., R. Tamarit, A. Viñals, J. Gómez, and C. Vives-Ferrándiz. 2009. Depredación sobre nidos, aves adultas y mamíferos por el lirón careto *Eliomys quercinus*. Galemys 21:3–11.

GODENA, G., F. D'ALONZO, AND M. CRISTALDI. 1978. Corrélations entre caryotypes et biotypes chez le lérot (*Eliomys quercinus*) et les autres rongeurs de l'ile Lipari. Mammalia 42:382–384.

GORNUNG, E., D. BIZZOCO, P. COLANGELO, AND R. CASTIGLIA. 2010. Comparative cytogenetic and genetic study of two Italian populations of the garden dormouse *Eliomys quercinus* L. (Sciuromorpha: Gliridae). Italian Journal of Zoology 77:137–143.

GOÜY DE BELLOCQ, J., H. FERTÉ, J. DEPAQUIT, J. L. JUSTINE, A. TILLIER, AND M. C. DURETTE-DESSET. 2001. Phylogeny of the Trichostrongylina (Nematoda) inferred from 28S rDNA sequences. Molecular Phylogenetics and Evolution 19:430–442.

GRAPHODATSKY, A. S., AND I. M. FOKIN. 1993. Comparative cytogenetics of Gliridae (Rodentia). Zoologicheskii Zhurnal 72:104–113.

GRILL, A., ET AL. 2009. Molecular phylogeography of European *Sciurus vulgaris*: refuge within refugia? Molecular Ecology 18:2687–2699.

GUARDIA, R. S. D., AND M. R. GIRELA. 1980. The chromosomes of three Spanish subspecies of *Eliomys quercinus* (Linnaeus). Genetica 51:107–109.

GUINDON, S., J. F. DUFAYARD, V. LEFORT, M. ANISIMOVA, W. HORDIJK, AND O. GASCUEL. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59:307–321.

HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

HASEGAWA, M., H. KISHINO, AND T. YANO. 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160–174.

HEWITT, G. M. 1999. Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society 68:87–112.

HOLDEN, M. E. 2005. Family Gliridae. Pp. 819–841 in Mammal species of the world: a taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, eds.). 3rd ed. Johns Hopkins University Press, Baltimore, Maryland.

HUELSENBECK, J., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

HÜRNER, H., ET AL. 2010. Mitochondrial phylogeography of the edible dormouse (*Glis glis*) in the western Palearctic region. Journal of Mammalogy 91:233–242.

INTERNATIONAL UNION FOR CONSERVATION OF NATURE (IUCN). 2011. Red list of threatened species. http://www.iucnredlist.org. Accessed 19 August 2011.

JOURNAL OFFICIEL DE LA COMMUNAUTÉ EUROPÉENNE, L38, 25ème année 10/02/1982 ISSN: 0378-7060

KELLER, L. F., AND D. M. WALLER. 2002. Inbreeding effects in wild populations. Trends in Ecology & Evolution 17:230–241.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120.

KOTSAKIS, T. 2003. Fossil glirids of Italy: the status of the art. Coloquios de Paleontología 1:335–343.

KOWALSKI, K. 2001. The Pleistocene rodents of Europe. Folia Quaternaria 72:1–389.

KRYŠTUFEK, B., AND R. KRAFT. 1997. Cranial variation and taxonomy of garden dormice (*Eliomys* Wagner, 1840) in the circum-Mediterranean realm. Mammalia 61:411–429.

LEONARD, A., G. DEKNUDT, AND M. MERGEAY. 1970. Chromosomes of the dormouse (*Eliomys quercinus* L.). Acta Zoologica et Pathologica Antverpiensia 50:55–60.

LIBOIS, R., AND M. G. RAMALHINHO. 2005. Phylogeography and postglaciation recolonisation of forest rodent species: the role of the southern Europe glacial refuges. Fundação para a Ciência e a Technologia (Ministério da Ciência e da Tecnologia, Lisboa), Lisboa, Project POCTI/BSE/36626/2000, Final Report:1–55.

LIBOIS, R., M. G. RAMALHINHO, AND R. ROSOUX. Zoogeography of the chromosomal races of the garden dormouse, *Eliomys quercinus*, in France. 2012 Acta Theriologica, 57 (4):313–320.

LIBRADO, P., AND J. ROZAS. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

LÓPEZ ANTOÑANZAS, R., AND G. CUENCA BESCÓS. 2002. The Gran Dolina site (Lower to Middle Pleistocene, Atapuerca, Burgos, Spain): new palaeoenvironmental data based on the distribution of small mammals. Palaeogeography, Palaeoclimatology, Palaeoecology 186:311–334.

LÓPEZ-GARCÍA, J. M., ET AL. 2010. Palaeoenvironmental and palaeoclimatic reconstruction of the latest Pleistocene of El Portalón site, Sierra de Atapuerca, northwestern Spain. Palaeogeography, Palaeoclimatology, Palaeoecology 292:453–464.

LUIKART, G., L. GIELLY, L. EXCOFFIER, J. D. VIGNE, J. BOUVET, AND P. TABERLET. 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. Proceedings of the National Academy of Sciences USA 98:5927–5932.

MACDONALD, D. W., AND P. BARRETT. 1993. Mammals of Britain and Europe. Harper Collins, London, United Kingdom.

MARKS, A. E., ET AL. 2002. Le gisement pléistocène moyen de Galeria Pesada (Estrémadure, Portugal): premiers résultats. Paléo 14:77–100.

MICHAUX, J. R., M. SARA, R. M. LIBOIS, AND R. F. MATAGNE. 1998. Is the woodmouse (*Apodemus sylvaticus*) of Sicily a distinct species? Belgian Journal of Zoology 128:211–214.

MILLER, G. S. (1912) Catalogue of the mammals of Western Europe. British Museum, London, 1019 p.

MITSAINAS, G. P., AND E. B. GIAGIA-ATHANASOPOULOU. 2005. Studies on the Robertsonian chromosomal variation of *Mus musculus domesticus* (Rodentia, Muridae) in Greece. Biological Journal of the Linnean Society 84:503–513.

MITSAINAS, G. P., M. T. ROVATSOS, I. KARAMARITI, AND E. B. GIAGIA-ATHANASOPOULOU. 2008. Chromosomal studies on Greek populations of four small rodent species. Folia Zoologica 57:337–346.

MONTGELARD, C., C. A. MATTHEE, AND T. J. ROBINSON. 2003. Molecular systematics of dormice (Rodentia: Gliridae) and the radiation of *Graphiurus* in Africa. Proceedings of the Royal Society of London, B. Biological Sciences 270:1947–1955.

MORITZ, C. 1994. Defining 'evolutionarily significant units' for conservation. Trends in Ecology & Evolution 9:373–374.

MURARIU, D., A. LUNGEANU, L. GAVRILA, AND C. STEPAN. 1985. Preliminary data concerning the study of the karyotype of *Eliomys quercinus* (Linnaeus, 1766) (Mammalia, Gliridae). Travaux du Museum d'Histoire Naturelle "Grigore Antipa" 27:325–327.

NACHMAN, M., AND P. MYERS. 1989. Exceptional chromosomal mutations in a rodent population are not strongly underdominant. Proceedings of the National Academy of Sciences USA 86:6666–6670.

NIEBERDING, C., R. LIBOIS, C. J. DOUADY, S. MORAND, AND J. R. MICHAUX. 2005. Phylogeography of a nematode (*Heligmosomoides polygyrus*) in the western Palearctic region: persistence of northern cryptic populations during ice ages? Molecular Ecology 14:765–779.

ORSINI, P. 1987. Le lérot. In Les mammifères en Corse. Ed. Parc nat. reg. Corse, Ajacciu, Gallia Préhistoire 26 supplement:99–101.

PESHEV, D., AND V. DELOV. 1995. Chromosome study of three species of dormice from Bulgaria. Hystrix—The Italian Journal of Mammalogy 6:151–153.

POLYAKOV, A. V., T. A. WHITE, R. M. JONES, P. M. BORODIN, AND J. B. SEARLE. 2011. Natural hybridization between extremely divergent chromosomal races of the common shrew (*Sorex araneus*, Soricidae, Soricomorpha): hybrid zone in Siberia. Journal of Evolutionary Biology 24:1393–1402.

POSADA, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

POVOAS, L., J. ZILHAO, J. CHALINE, AND P. BRUNET-LECOMTE. 1992. La faune de rongeurs du Pléistocene supérieur de la grotte de Caldeirao (Tomar, Portugal). Quaternaire 3:40–47.

RAMALHINHO, M. G., AND R. LIBOIS. 2001. The karyotype of the Formentera Island garden dormouse, *Eliomys quercinus ophiusae*. Belgian Journal of Zoology 131:83–85.

RAMALHINHO, M. G., AND R. LIBOIS. 2005. Karyotype of dormice *Eliomys quercinus* from Tirol (Austria). Acta Theriologica 50:133–136.

RENAUD, P. 1938. La formule chromosomiale chez sept espèces de Muscardinidae et Microtinae indigènes. Revue Suisse de Zoologie 45:349–385.

REYES, A., G. PESOLE, AND C. SACCONE. 1998. Complete mitochondrial DNA sequence of the fat dormouse, *Glis glis*: further evidence of rodent paraphyly. Molecular Biology and Evolution 15:499–505.

RONCHITELLI, A., ET AL. 2011. The Grotta Grande of Scario (Salerno, Italy): archaeology and environment during the last interglacial (MIS 5) of the Mediterranean region. Quaternary International 231:95–109.

RONQUIST, F., AND J. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

SACCHERI, I., M. KUUSSAARI, M. KANKARE, P. VIKMAN, W. FORTELIUS, AND I. HANSKI. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494.

SALAZAR-BRAVO, J., J. W. DRAGOO, D. S. TINNIN, AND T. L. YATES. 2001. Phylogeny and evolution of the Neotropical rodent genus *Calomys*: inferences from mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution 20:173–184.

SEKEROĞLU, V., H. KEFELIOĞLU, AND Z. A. SEKEROĞLU. 2011. G- and C-banded karyotypes of hazel dormouse, *Muscardinus avellanarius trapezius* (Mammalia: Rodentia) in Turkey. Turkish Journal of Zoology 35:375–379.

SESÉ, C., AND P. VILLA. 2008. Micromammals (rodents and insectivores) from the early Late Pleistocene cave site of Bois Roche (Charente, France): systematics and paleoclimatology. Geobios, 41:399–414.

TAMURA, K., D. PETERSON, N. PETERSON, G. STECHER, M. NEI, AND S. KUMAR. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28:2731–2739.

TRANIER, M., AND F. PETTER. 1978. Les relations d'*Eliomys tunetae* et de quelques autres formes de Lérots de la région méditerranéenne (Rongeurs, Muscardinidés). Mammalia 42:349–354.

TRAVESET, A., ET AL. 2009. A review on the effects of alien rodents in the Balearic (western Mediterranean Sea) and Canary Islands (eastern Atlantic Ocean). Biological Invasions 11:1653–1670.

VEYRUNES, F., ET AL. 2010. Mitochondrial and chromosomal insights into karyotypic evolution of the pygmy mouse, *Mus minutoides*, in South Africa. Chromosome Research 8:563–574.

VIGNE, J. D., AND J. A. ALCOVER. 1985. Incidence des relations historiques entre l'homme et l'animal dans la composition actuelle du peuplement amphibien, reptilien et mammalien des îles de Méditerranée occidentale. Pp. 79–91 in 110e Congrès national des Sociétés savantes, Montpellier, France.

VILLA, P., ET AL. 2010. The archaeology and paleoenvironment of an Upper Pleistocene hyena den: an integrated approach. Journal of Archaeological Science 37:919–935.

VUJOSEVIC, M., M. G. FILIPPUCCI, J. BLAGOJEVIC, AND B. KRYŠTUFEK. 1993. Evolutionary genetics and systematics of the garden dormouse, *Eliomys* Wagner, 1840. IV: karyotype and allozyme analyses of *E. quercinus dalmaticus* from Yugoslavia. Bollettino di Zoologia 60:47–51.

WHITTAKER, R. J., AND J. M. FERNÁNDEZ-PALACIOS. 2007. Island biogeography: ecology, evolution, and conservation. Oxford University Press, Oxford, United Kingdom.

ZIMA, J., M. MACHOLÁN, M. ANDERA, AND J. CERVENY. 1997. Karyotypic relationships of the garden dormouse (*Eliomys quercinus*) from central Europe. Folia Zoologica—Praha 46:105–108.

ZIMA, J., M. MACHOLÁN, AND M. G. FILIPPUCCI. 1995. Chromosomal variation and systematics of myoxids. Hystrix 6:63–76.

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Appendix I

Geographic distribution, karyotype, and references for sampled individuals of *Eliomys* sp. The karyotype of each sequenced individual is known, either based on direct previous experimentation for 52 individuals of *E. quercinus* and 4 individuals of *E. melanurus*, or based on the comparison with the karyotype of individuals from the same region for the 10 additional *E. quercinus* sampled in Corsica ("Italian" 48-chromosome race—Orsini 1987), in Belgium (50-chromosome race—Leonard 1970), and in Savoie and Aoste (54-chromosome race—Libois et al., 2012).

Species and clade	Country	Population	Tissue sample	Karyotype	Haplotype	Harvester (initials, last name)—karyotype references
E. quercinus						
Alpine	France	Hautes-Alpes	2421	54 Alpine	FA1	R., Fl., and Fr. Libois—Libois et al. 2012
			2435	54 Alpine	FA2	idem
			2440	54 Alpine	FA3	idem
		Savoie	20100924001	Unknown	FA4	R. Libois and R. Callejon—Libois et al. 2012
			20100924002	Unknown	FA4	idem
			20100924006	Unknown	FA4	idem
			20100924007	Unknown	FA4	idem
			2010092604	Unknown	FA4	idem
			2420	54 Alpine	FA5	R., Fl., and F. Libois—Libois et al. 2012
			2436	54 Alpine	FA6	idem
	Italy	Aoste	2454	54 Alpine	IA1	M. G. Filippucci—Filippucci et al. 1988a
			2457	54 Alpine	IA1	idem
			2479	54 Alpine	IA1	idem
			2010092805	Unknown	IA2	R. Libois and R. Callejon—Libois et al. 2012
			2011092904	54 Alpine	IA2	R. Libois and R. Callejon—Libois et al. 2012
		Friuli-Venezia Giulia	2453	52 Alpine	IA3	M. G. Filippucci—Filippucci et al. 1988a
		Liguria-Piedmont	2428	54 Alpine	IA4	R., Fl., and Fr. Libois—Libois et al. 2012
			2478	54 Alpine	IA5	M. G. Filippucci—Filippucci et al. 1988a
		Trentino Alto Adige	2425	52 Alpine	IA6	R. Libois and M. G. Ramalhinho—Filippucci et al. 1988a
			2426	52 Alpine	IA6	idem
		Veneto	2455	52 Alpine	IA7	M. G. Filippucci—Filippucci et al. 1988a
Iberian	France	Charente	2417	48 Iberian	Ib1	R. Rosoux and R. Libois—Libois et al. 2012
		Lozère	523	48 Iberian	Ib2	idem
			2450	48 Iberian	Ib2	idem
			2447	48 Iberian	Ib3	C. Nappée and R. Libois—Libois et al., 2012
		Pyrénées-Orientales	20090604	48 Iberian	Ib4	R. Libois and R. Fons—Libois et al. 2012
			1446B	48 Iberian	Ib1	R. Fons and R. Libois—Libois et al. 2012
			2438	48 Iberian	Ib1	R. Libois and M. G. Ramalhinho—Filippucci et al. 1988a
			2486	48 Iberian	Ib1	idem
			2445	48 Iberian	Ib5	idem
	Portugal	Portugal	2424	48 Iberian	Ib6	M. G. Ramalhinho—Libois et al. 2012
			2432	48 Iberian	Ib7	idem
			2448	48 Iberian	Ib8	M. G. Ramalhinho and R. Libois—Libois et al. 2012
			2476	48 Iberian	Ib8	idem
	Spain	Formentera (Balearic Islands)	2471	48 Iberian	Ib9	R. Libois and C. Hallet—Ramalhinho and Libois 2001
Italian	Croatia	Dalmatia	2464	48 Italian	It1	M. G. Filippucci—Filippucci et al. 1988a
			2465	48 Italian	It1	idem
			2466	48 Italian	It1	idem
	France	Corsica	2418	Unknown	It2	M. Salotti—pers. comm., University of Corse (Corte).
	Italy	Sardinia	1442B	50 Sardinian	It3	R. Libois and M. G. Ramalhinho—Libois et al. 2012
			1443B	50 Sardinian	It4	idem
			1444B	50 Sardinian	It5	idem
			2427	50 Sardinian	It2	idem
			2446	50 Sardinian	It2	idem
			2487	50 Sardinian	It2	idem

Appendix I.—Continued.

Species and clade	Country	Population	Tissue sample	Karyotype	Haplotype	Harvester (initials, last name)—karyotype references
		Umbria	2451	48 Italian	It6	M. G. Filippucci—Filippucci et al. 1988a
			2452	48 Italian	It7	idem
			2456	48 Italian	It8	idem
			2473	48 Italian	It6	idem
Western European	Austria	Vorarlberg (east)	2467	50 northern	WE1	M. G. Filippucci—Filippucci et al. 1988
	Belgium	Belgium (east)	2429	50 northern	WE2	A. M. Massin and R. Libois—Libois et al. 2012
			2430	50 northern	WE2	A. Laudelout and R. Libois—Libois et al. 2012
			2011032501	Unknown	WE3	R. Libois, F. Bssaibis, and G. C. L. Perez—Libois et al. 2012
			2011032502	Unknown	WE2	idem
			20110325LB	Unknown	WE2	idem
	France	Haut-Rhin (east)	1445B	50 northern	WE4	R. Libois—Libois et al. 2012
		Loiret (west)	2437	50 northern	WE4	R. Rosoux—Libois et al. 2012
		Normandy (west)	2422	50 northern	WE5	F. Leboulenger and R. Libois—Libois et al. 2012
		Vendée (west)	2423	49 Iberian/northern	WE6	H. des Touches and R. Libois—Libois et al. 2012
			2434	48 Iberian	WE6	R. Rosoux and R. Libois—Libois et al., 2012
			2449	48 Iberian	WE6	H. des Touches and R. Libois—Libois et al. 2012
	Germany	Rhineland-Palatinate (east)	2468	50 northern	WE7	M. G. Filippucci—Filippucci et al. 1988a
E. melanurus						
Morocco	Morocco	Cape Spartel	2485	46 Moroccan	Mo1	R. Libois and M. G. Ramalhinho—Libois et al. 2012
		Ouezzane	2475	46 Moroccan	Mo2	M. G. Filippucci—Filippucci et al. 1988a
Morocco–Negev	Israel	Negev Desert	2462	48 Israeli	Neg	idem
	Morocco	Cape Spartel	2463	46 Moroccan	Mo3	idem