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Mitochondrial phylogeography of the edible dormouse (*Glis glis*) in the western Palearctic region

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This study describes in detail the phylogeographic pattern of the edible dormouse (*Glis glis*) a European rodent with pronounced hibernating behavior. We used sequences of 831 base pairs of the mitochondrial DNA cytochrome-*b* gene from 130 edible dormice collected at 43 localities throughout its distribution. Our results reveal presence of 3 main haplogroups: Sicilian, South Italian (restricted to the Calabrian region), and European (a widespread lineage corresponding to all remaining western, central, and eastern European populations). Examination of paleontological data confirms refugial regions for *G. glis* in the 3 Mediterranean peninsulas, although overall low genetic diversity is found. The low diversity of the European lineage is probably the result of a recent expansion (dated around 2,000 years ago) from a single refugium. Other factors, such as the ecological constraints on the species, may have caused genetic bottlenecks that reinforced the low genetic variability of *G. glis*. This work could have important implications for strategies to conserve the edible dormouse by defining important areas for their conservation. DOI: 10.1644/08-MAMM-A-392R1.1.

Key words: dormouse, Europe, glacial refugia, *Glis glis*, mitochondrial DNA, phylogeography, postglacial colonization

Quaternary climatic oscillations have played a major role in shaping the present geographical distribution of species, including their genetic structure and diversity (Avise 1994). This has resulted in the extinction of northern populations of many species during ice ages, followed by subsequent northward expansions from refugia during interglacial periods (Hewitt 1996, 1999, 2000; Taberlet et al. 1998). Refugia for European small mammals were located mainly in the Mediterranean, the Urals, and the Caucasus–Carpathian region (Hewitt 1996; Jaarola and Searle 2002; Taberlet et al. 1998), with additional refugia in central Europe (Bilton et al. 1998; Brunhoff et al. 2003). Avise (1994) and Hewitt (1996) postulated that rapid expansion from refugial populations involved periodic bottlenecking with progressive loss of allelic diversity. This would result in lower genetic diversity in populations present in the more recently colonized places. In contrast, it is expected that populations that remain in the Mediterranean refugia would be affected less by climatic changes and more diverse genetically. Moreover, the isolation of populations of many species in separate southern regions during ice ages could have resulted in allopatric differentiation into several genetic groups that recolonized the western Palearctic region at the end of the last ice age 11,600 years ago. This model has been used extensively to interpret the history of various organisms during the Pleistocene glaciations. There are several large-scale phylogeographical studies

associated with the refugium theory in rodents of the Northern Hemisphere, including the field vole (*Microtus agrestis*—Jaarola and Searle 2002), the common vole (*Microtus arvalis*—Haynes et al. 2003), the root vole (*Microtus oeconomus*—Brunhoff et al. 2003), the bank vole (*Myodes glareolus*—Deffontaine et al. 2005), and the woodmouse (*Apodemus sylvaticus*—Michaux et al. 2003).

Although glirids (dormice) represent 1 of the oldest rodent families (Daams and De Bruijn 1994) and were once widespread and diverse, they currently have a relatively limited distribution. Nine extant genera comprise the family. One species of dormouse (*Glirulus japonicus*) occurs in Japan, and several species of *Graphiurus* inhabit large areas of Africa; otherwise, dormice are essentially a Palearctic group (P. Morris, pers. comm.). In recent times natural scarcity has been exacerbated by anthropogenic environmental damage, and several species are now regarded as rare or endangered. Many dormouse species are profound hibernators. Their sensitivity to both climate and other environmental factors means that dormice are important bioindicators of environmental change (P. Morris, Ascot, United Kingdom, pers. comm.).

Despite their importance as potential bioindicators, glirids have been the focus of few phylogeographic studies to date. The impact of glacial cycles on rodent species and the ability of certain temperate species to persist in portions of their current range during cold periods depend on their physiological tolerance to climate change (Hewitt 2004). Obligatory thermophilous species are more affected by cold phases than are cold-tolerant organisms, thus the evolutionary history of glirids may show significant differences compared to other species.

The aim of this study is to infer the phylogeography of the edible dormouse (*Glis glis* Linnaeus, 1766). The distribution of this hibernating mammal coincides mainly with the deciduous forests of Europe and adjacent regions of the Near East. It occurs from France and the Spanish Pyrenees to the Volga River, northern Iran, and Latvia (Krystufek 1999). It is present on the islands of Sardinia, Corsica, Sicily, Crete, and Corfu, but also on small islands in the Thyrrenian (Elba, Asinara, and Salina), Adriatic, and Aegean seas (Sarà 1998). *G. glis* is rare over much of its geographic range, particularly in the north (Krystufek 1999). This study was conducted to investigate the following questions: Does the phylogeographical structure of *G. glis* match that of other European rodents? Is phylogeographical structure of *G. glis* in agreement with a late glacial or postglacial model of expansion and, if so, where were the glacial refugia?

Materials and Methods

Samples and laboratory procedures.—A total of 130 *G. glis* taken from 43 localities (1–9 samples per population) from throughout its geographical range was analyzed (Fig. 1; Appendix I). These specimens were obtained from collaborators, museums, and our own fieldwork. All samples used in the present study were skin samples stored in ethanol, except for the Hungarian samples, which were dried hairs. Genomic DNA was extracted using the DNeasy Tissue kit (Qiagen Inc., Valencia, California) following the manufacturer's instructions. The mitochondrial cytochrome-*b* (*Cytb*) gene was amplified using specific primers designed for *G. glis*: FGLIS1 (5′-CAGCTTGATGAAACTTTGG-3′) and RGLIS1 (5′-CCAATTCATGTGAGGGTG-3′). Amplifications were carried out following the protocol of Michaux et al. (2003) and performed in a Labover PTC100 thermal cycler (MJ Research, Watertown, Massachusetts) employing 39 cycles (30 s at 94°C, 1 min at 52°C, and 2 min at 68°C) with a final extension cycle of 10 min at 68°C. All the sequencing procedures were performed by Macrogen Inc. (Seoul, Korea). Sequences were aligned using ClustalW algorithm in BioEdit 7.0.5.2 (Hall 1999).

Data analysis.—Phylogenetic reconstructions were performed to detect the relative positions of the observed lineages using the maximum-likelihood algorithm (Felsenstein 1981) implemented in the PHYML program (Guidon and Gascuel 2003). We used MODELTEST version 3.0 (Posada and Crandall 1998) to determine the most suitable model of DNA substitution for the *Cytb* data set. The robustness of phylogenetic trees was assessed by bootstrap resampling (Felsenstein 1985). A Bayesian phylogeny reconstruction approach (Yang and Rannala 1997) also was used, as implemented in MRBAYES 2.01 (Huelsenbeck et al. 2001). Metropolis-coupled Markov chain Monte Carlo sampling was performed with 5 chains run for 1 million iterations, using default model parameters as starting values. Bayesian posterior probabilities were taken from the 50% majority rule consensus of trees sampled every 100 generations, discarding the trees obtained before the chains became stationary.

Haplotype networks can portray more effectively relationships among sequences for populations with low sequence diversity (Crandall and Templeton 1993), so a minimum spanning network was constructed using the MINSPNET algorithm available in the Arlequin 2.0 program (Schneider et al. 2000). Haplotype diversity (h), nucleotide diversity (π—Nei 1987), and their standard deviations (Tajima 1993) were estimated using DnaSP 4.0 program (Rozas et al. 2003). The genetic structure of populations was examined using an analysis of molecular variance (AMOVA) performed in Arlequin. The AMOVA was conducted at 3 hierarchical levels of population subdivisions: among genetic groups (corresponding to the observed lineages), among populations within each genetic group (24 populations were defined according to geographical data), and within each population. The significance of these parameters was estimated by 10,000 permutations of the distance matrix.

Demographic histories of different haplogroups of *G. glis* were inferred 1st by a pairwise mismatch distribution analysis between individuals (Rogers and Harpending 1992) computed under a population growth-decline model in DnaSP 4.0 (initial $\theta = 2$, final $\theta = 200$, $\tau = 3$). Multimodal distributions are consistent with demographic stability, whereas sudden expansion would generate a unimodal pattern (Slatkin and Hudson 1991). The timing of demographic expansion can be estimated

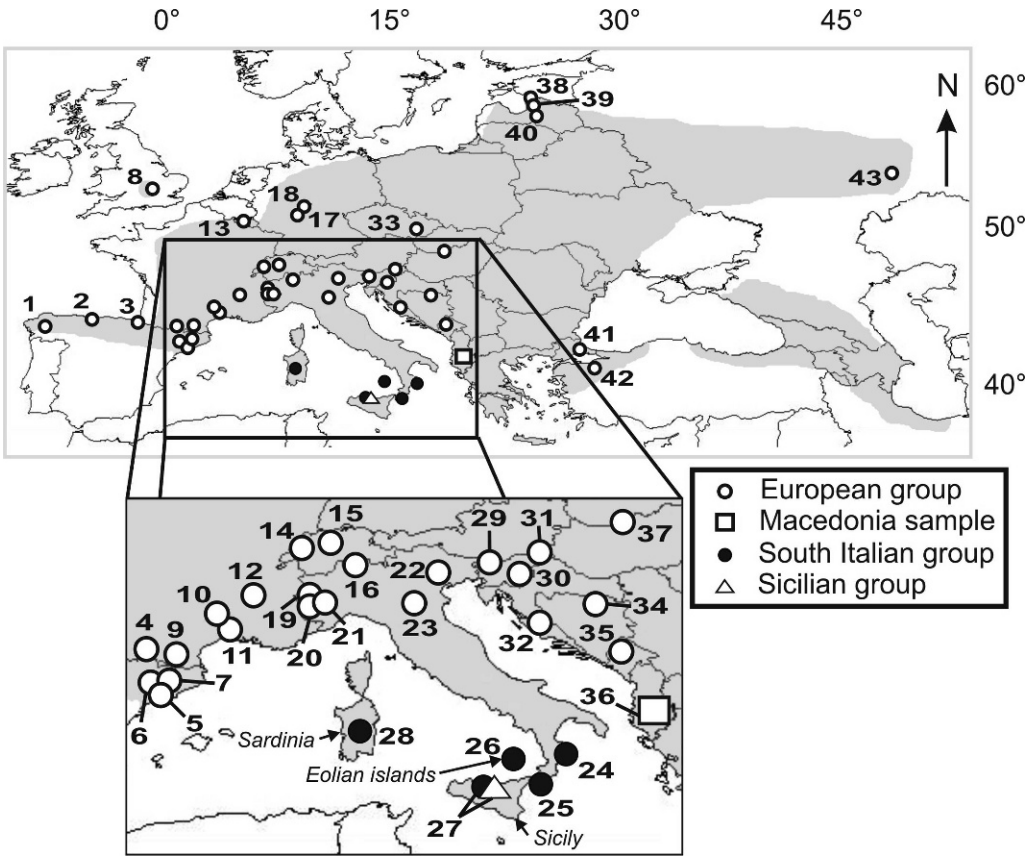

Fig. 1.—Geographical distribution of samples of edible dormouse (*Glis glis*) examined in this study. Symbols indicate the 4 genetic haplogroups identified in the analysis of mitochondrial cytochrome-*b* sequences. The shaded region indicates the known distribution of *G. glis* (Krystufek 1999; Morris 2004).

by the mode of mismatch distribution (τ) expressed as $\tau = 2\mu t$, where t is the expansion time in number of generations and μ is the mutation rate for the entire sequence (Rogers 1995). Population history also was inferred by testing departure from neutrality using R_2 (Ramos-Onsins and Rozas 2002) in DnaSP and Fu's F_S statistic (Fu 1997) in Arlequin. Both R_2 and F_S are powerful tests used to detect population expansion under assumptions of neutrality (Fu 1997; Ramos-Onsins and Rozas 2002).

We used the McDonald–Kreitman test (McDonald and Kreitman 1991) in DnaSP to explore if natural selection acted on our *Cytb* data set. We used a Fisher's exact test to determine whether the ratio of synonymous to nonsynonymous substitutions differs between 2 categories: polymorphisms that are variable within *G. glis* and the hazel dormouse (*Muscardinus avellanarius*), and polymorphisms that distinguish these 2 species (i.e., fixed differences).

Relative-rate tests and an approximate time of divergence between the observed mitochondrial DNA (mtDNA) lineages were calculated as explained in Michaux et al. (2003). The calibration point was derived from paleontological data reported by Montgelard et al. (2003), in which the divergence time between *Eliomys quercinus* and *E. melanurus* was estimated at approximately 7 ± 0.9 million years ago (mya).

Results

Phylogenetic and phylogeographic analysis.—Sixteen haplotypes were identified among the 130 specimens of *G. glis* examined. All sequences have been deposited in GenBank (accession numbers FM160651–FM160665, FM160733, and FM160734). The complete data matrix consisted of these 16 haplotypes plus sequences from 3 specimens of *E. quercinus*, 2 of *E. melanurus*, and 1 specimen of *M. avellanarius* as outgroups. Outgroups were chosen on the basis of a molecular phylogenetic study of the Gliridae (Montgelard et al. 2003). The matrix included 831 base pairs for each specimen, of which 72 sites were variable and 19 were informative for parsimony. The average transition–transversion ratio was 3.9, and nucleotide frequencies were 31.9%, 27.1%, 28.4%, and 12.6% for T, C, A, and G, respectively. Maximum-likelihood analyses were performed using the GTR + Gamma model suggested for the data by the Akaike information criterion in MODELTEST, with the proportion of invariable sites equal to 5.3719 and the gamma distribution shape parameter equal to 0.3470.

The maximum-likelihood phylogenetic tree (Fig. 2) and Bayesian tree (not illustrated) showed identical topologies. The 16 haplotypes of *G. glis* fell into 3 major lineages: the 1st comprising some individuals from Sicily (lineage 1; maximum-likelihood bootstrap support = 100%; Bayesian posterior probability = 0.99), the 2nd corresponding to populations from

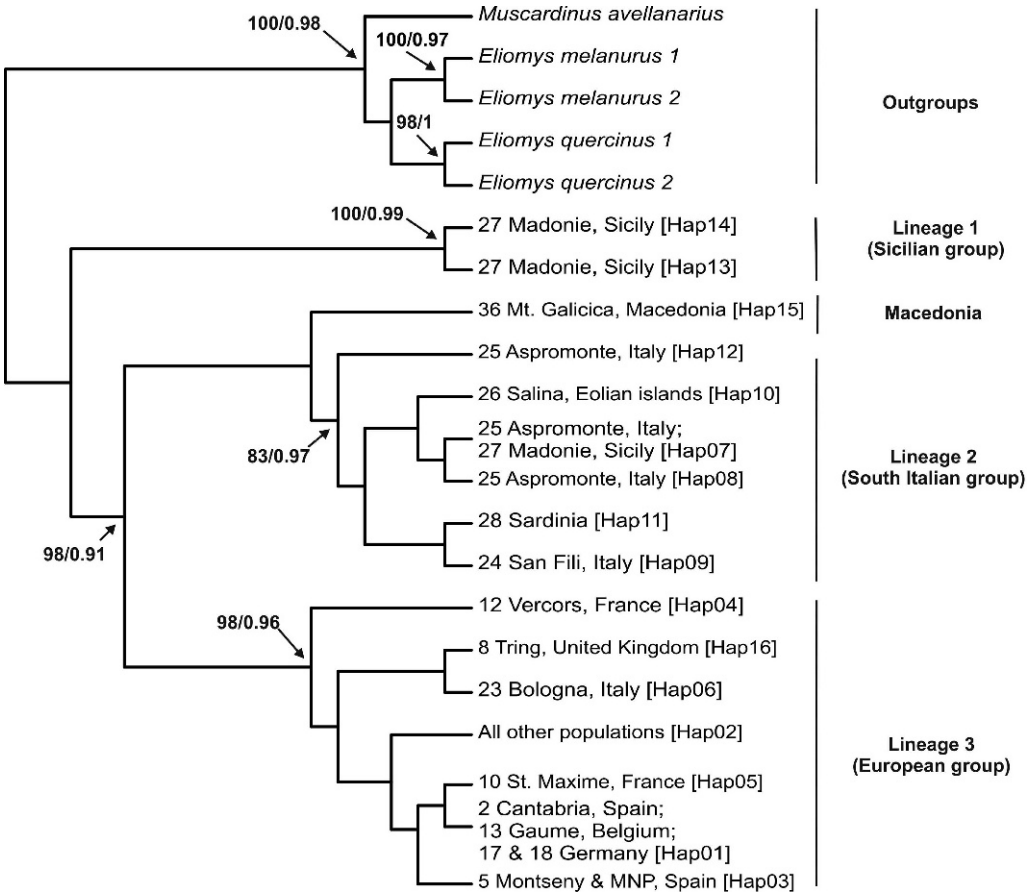

Fig. 2.—Maximum-likelihood tree for the 16 haplotypes identified in edible dormouse (*Glis glis*). Locality numbers (from Fig. 1 and Appendix I) are indicated before locality names, and haplotype designations (Appendix I) are indicated in brackets following locality names. Numbers at nodes indicate maximum-likelihood bootstrap values followed by Bayesian posterior probability values greater than 50% or 0.5, respectively.

southern Italy, including a few Sicilian specimens (lineage 2; maximum-likelihood bootstrap support = 83%; Bayesian posterior probability = 0.97), and the 3rd comprising populations from northern Italy and western, central, and eastern Europe (lineage 3; maximum-likelihood bootstrap support = 98%; Bayesian posterior probability = 0.96). The Macedonian specimen is separated from lineage 3, but with low support (maximum-likelihood bootstrap support = 48%; Bayesian posterior probability = 0.45). The genetic divergence among these 3 lineages is low, varying from 0.5% to 1.5% Kimura 2-parameter distance (Kimura 1980).

The minimum spanning network (Fig. 3) also showed these 3 main haplogroups, with the Macedonian specimen again separated from them. A minimum of 8 mutational steps is found between each of the 3 major groups. The South Italian group and the Sicilian group are highly differentiated (28 mutational steps; Fig. 3), and the European group shows a starlike topology (Fig. 3), characteristic of recently expanding groups (Avise 2000).

Analysis of genetic diversity and differentiation.—To assess whether genetic diversity was higher within putative glacial refugia, the European group (lineage 3) was divided into 4 subgroups (Table 1): the 1st corresponding to the Iberian and southern France populations (sublineage 3.1), the 2nd to the Balkan populations (sublineage 3.2), the 3rd to northern Italy (sublineage 3.3), and the 4th to all other European populations (sublinage 3.4). All lineages and sublineages showed low nucleotide diversity (π between 0.0005 and 0.003). Animals from southern Italy (lineage 2) showed the highest level of genetic diversity ($\pi = 0.003$), and the AMOVA showed that most of the mtDNA variation (95.3%) was between, rather than within, the 3 main lineages (Table 1).

Analysis of demographic history and influence of selection.—The mismatch distribution showed no significant signature of population expansion or stability for the 3 major lineages. However, tests of neutrality were significant for the European group ($F_S = -6.83$, $P = 0.003$; $R_2 = 0.017$, $P = 0.000$), a strong indication of population expansion also indicated by the minimum spanning network. Tests of neutrality for the South Italian group were not significant ($F_S = -1.54$, $P = 0.09$; $R_2 = 0.113$, $P = 0.05$), suggesting demographic stability of this population. The time expansion for the European group was estimated at approximately 2,000 years ago. The McDonald–Kreitman test showed no significant evidence of selection on the *Cytb* gene of *G. glis*.

Divergence time.—The relative rate tests indicate no significant rate heterogeneity for both synonymous and nonsynonymous substitutions in the *Cytb* gene between the

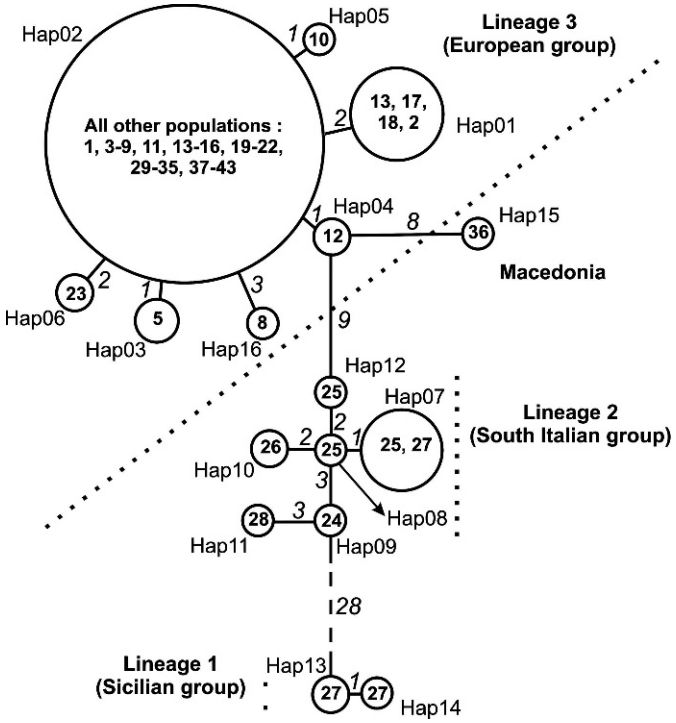

Fig. 3.—A minimum spanning network constructed using the 16 mitochondrial cytochrome-*b* haplotypes identified in edible dormouse (*Glis glis*). Haplotype designations (Appendix I) are indicated next to each circle. Locality numbers (see Fig. 1) for specimens possessing each haplotype are indicated inside the circles. Size of circles is proportional to number of individuals possessing that haplotype (smallest circle, $n = 1$; largest circle, $n = 95$). Length of lines between circles is roughly proportional to estimated number of mutational steps between the haplotypes (actual estimated number of steps indicated on the lines). Four of 7 specimens with the hap07 were from locality 27 and 3 were from locality 25 (see Fig. 1).

lineages of *E. quercinus*. The mean Kimura 2-parameter distance between *E. quercinus* and *E. melanurus*, which according to fossil evidence diverged 7 mya (Montgelard et al. 2003), is 7.2%. This value corresponds to a rate of 1% Kimura 2-parameter distance per million years. When this rate is applied to the major divergences within *G. glis* (taking into account the correction for ancestral mtDNA polymorphism), separation between the European and South Italian groups is estimated to have occurred 0.48 mya, and separation between the South Italian and Sicilian groups is estimated at 0.97 mya.

Discussion

Genetic structure of G. glis.—In contrast to the other studies of European rodents, which reported high levels of genetic divergence (Brunhoff et al. 2003; Cosson et al. 2005; Deffontaine et al. 2005, 2009; Dubey et al. 2007; Fink et al. 2004; Jaarola and Searle 2002, 2004; Kotlik et al. 2006; Michaux et al. 2003, 2005; Neumann et al. 2005), our results show very low genetic variability in *G. glis*. The evolutionary trees and haplotype network divide *G. glis* into 3 well-supported genetic lineages that have nonoverlapping geographical distributions (with the exception of Sicilian animals occurring in 2 different haplogroups). The European lineage is widespread from northern Spain, northern Italy, and Turkey in the south, to Latvia in the north, and to the Volga River (Russia) in the east. The South Italian group is limited to central and southern Calabria, the Eolian islands, Sardinia, and Sicily. The 3rd lineage corresponds to 3 individuals from Sicily.

Glacial refugia.—The South Italian group of *G. glis* shows higher nucleotide and haplotype diversity compared to populations in northern Italy, Iberia, the Balkans, and other European populations. Because fossil remains of *G. glis* have been reported from Calabria (Italy) during the late glacial periods (12,100 years ago—Fiore et al. 2004), it seems likely that this region played a role as a glacial refugium for this species. This refuge located close to the Mediterranean Sea is in agreement with the pattern of refugia that has been reported previously for other rodent species (Deffontaine et al. 2005; Haynes et al. 2003; Jaarola and Searle 2002; Michaux et al. 2003, 2005).

The Iberian and Balkans populations of *G. glis* are characterized by especially low genetic diversity, despite these 2 regions possibly having served as refugia during the culmination of the last glacial maximum (Sommer and Nadachowski 2006). It is possible that a genetic bottleneck occurred in *G. glis* during this period in the Iberian Peninsula (and perhaps the Balkans), which is thought to have occurred in *A. sylvaticus* (Michaux et al. 2003). Genetic bottlenecks

Table 1.—Genetic variability observed within the main genetic groups of edible dormouse (*Glis glis*).

Lineages and their subgroups	*n*	No. haplotypes	Nucleotide diversity ($\pi \pm SD$)	Haplotype diversity ($h \pm SD$)
Sicilian group (lineage 1)	3	2	0.001 ± 0.001	0.67 ± 0.31
South Italian group (lineage 2)	13	6	0.003 ± 0.002	0.72 ± 0.13
European group (lineage 3)	113	7	0.001 ± 0.0006	0.29 ± 0.05
European sublineage 3.1 (southern France, Spain)	29	4	0.0005 ± 0.0005	0.31 ± 0.11
European sublineage 3.2 (Slovenia, Croatia, Turkey, Bosnia and Herzegovina, Macedonia, Montenegro)	27	2	0.001 ± 0.0009	0.07 ± 0.07
European sublineage 3.3 (northern Italy: Torino-Viu, Druento, Asti, Bologna, Asiago)	12	2	0.001 ± 0.0008	0.30 ± 0.15
European sublineage 3.4 (remainder of European group)	45	4	0.001 ± 0.0008	0.40 ± 0.08

could have been associated with increased aridity during cold periods (Tzedakis 1994), which probably influenced the distribution of suitable habitat (woodlands) for *G. glis*, leading to fragmentation of populations. However, it also is possible that sampling bias in this study could have artificially depressed measures of genetic diversity in the Balkans. Most of our samples come from the northern Balkans (e.g., Croatia and Slovenia), but the southern Balkans (e.g., Greece) could actually be an undetected refugium with levels of genetic divergence and diversity as high as in southern Italy. This hypothesis is supported by evidence from the single individual from Macedonia, which is similarly divergent from both the European and the Italian clades. Additional sampling from the southern Balkans could reveal greater diversity and thus evidence for possible glacial refugia in this region.

The Italian and Sicilian populations of G. glis.—Our study showed slightly higher genetic diversity in populations of *G. glis* from the Italian peninsula, where populations from northern Italy are separated from the South Italian group (Fig. 1), and divergence seems to appear between central Calabria (locality 24) and southern Calabria (locality 25; Fig. 3). These results suggest possible refugia within refugia in Italy, and several studies (Canestrelli et al. 2006, 2007; Podnar et al. 2005; Santucci et al. 1996; Ursenbacher et al. 2006) have shed light on possible multiple glacial refugia in the Italian peninsula. One example involves the beech tree (*Fagus sylvatica*—Magri et al. 2006), which is a dominant species in several dormouse habitats and particularly important to *G. glis*. Paleogeographic studies suggest the separation of central and southern Calabria from the rest of Italy in the past (reviewed by Santucci et al. 1996). However, in our case, the hypothesis of refugia within refugia would need to be confirmed by better sampling in the Calabria region.

Sicilian populations of *G. glis* with haplotypes hap13 and hap14 show evidence of isolation from Italian mainland populations in the past (Fig. 2). The same was observed in the nematode *Heligmosomoides polygyrus* (Nieberding et al. 2005) and the woodmouse *A. sylvaticus* (Michaux et al. 2003). Our results showed 2 genetic lineages of *G. glis* in Sicily (1 from lineage 1 and 1 from lineage 2; Fig. 3), a pattern also observed in the green toad (*Bufo viridis*—Stöck et al. 2008). Because the 2 Sicilian lineages of *G. glis* are not sister groups (Fig. 2), the simplest hypothesis to explain their presence in Sicily would be to postulate an ancient ancestral population of *G. glis* (lineage 1, hap13 and hap14) isolated in Sicily, followed by more recent colonization by a mainland population (lineage 2, hap07). A similar hypothesis has been proposed for *A. sylvaticus* (Michaux et al. 2003) and *H. polygyrus* (Nieberding et al. 2005). The isolation of insular populations of *A. sylvaticus* and *H. polygyrus* appears to have occurred during the same period, around 1 mya (Nieberding et al. 2005). Similar estimations of isolation also were proposed for the green toad (Stöck et al. 2008), except that for this species, the ancestors probably originated in North Africa. Initial isolation of these animal lineages in Sicily could have been caused by geological phenomena, such as sea level changes, that occurred in the early part of the middle Pleistocene. More recent human traffic between mainland Italy and Sicily could have facilitated introduction into Sicily of hap07, which is shared with Calabrian animals from Aspromonte (locality 27; Fig. 1). During the Neolithic, transportation of chert and obsidian across the Tyrrhenian Sea may have given dormice the opportunity to spread into the islands (Carpaneto and Cristaldi 1995). This hypothesis is supported by presence of mandibles of *G. glis* at a Neolithic site in Sicily (Sarà 2000), and a similar hypothesis of a recent invasion of Sicily by animals from the mainland was proposed for the green toad by Stöck et al. (2008).

Presence of an ancient lineage of *G. glis* in Sicily is not supported by available paleontological evidence (Petruso 2000), but better paleontological (and genetic) sampling from the islands in this region should improve understanding of the phylogeographic history of the dormouse in this region. Regardless, our results indicate that the Calabria region of southern Italy and Sicily could be ''hot spots'' of intraspecific biodiversity for *G. glis*. These regions would thus deserve attention when selecting Evolutionary Significant Units (Moritz 1994) for conservation of this species.

Postglacial expansion.—Expansion from a single refugium is the best explanation for the observed pattern of widespread genetic homogeneity in *G. glis* in Europe (Table 1). This scenario is supported by the starlike topology of the European lineage (Figs. 2 and 3) and by Fu's F_S and R_2 neutrality tests. Paleontological evidence attests to presence of *G. glis* in the 3 Mediterranean peninsulas (Iberia, the Balkans, and southern Italy) at the appropriate time in the past. The high endemism of the South Italian group suggests that it did not contribute to the European postglacial recolonization. Instead, it appears that recolonization of Europe by *G. glis* came from populations in northern Italy, Iberia, or the Balkans. Nothing more precise can be concluded from our results.

Genetic homogeneity in G. glis in Europe.—In contrast to other phylogeographic studies of rodents, our results show widespread genetic homogeneity in the European lineage of *G. glis*. Low genetic diversity and absence of genetic structure in European populations of *G. glis* could be explained by serial bottlenecks and progressive loss of allelic diversity during a rapid range expansion (Avise 2000; Hewitt 1999). However, our dating of this expansion, estimated here at 2,000 years ago, is inconsistent with paleontological data, which show presence of *G. glis* in central Europe as much as 10,000 years ago (Boessneck 1978; Storch 1987). Nonetheless, dates based on rates of genetic divergence are subject to much variation and should be interpreted with caution (Ho et al. 2005). Recent and rapid expansion of *G. glis* into Europe may have been linked with the rapid spread of oak forests (approximately 380 m per year) into northern Europe during the Holocene (Kremer et al. 2002). It also is possible that humans contributed to the rapid spread of *G. glis* across Europe. Edible dormice were a delicacy for ancient Romans, and the Roman habit of introducing both domestic and wild animals throughout the empire is well documented (Carpaneto and Cristaldi 1995).

Ancestral populations of *G. glis* may have experienced a mitochondrial selective sweep leading to the invasion of northern Europe by animals more resistant to cold (Avise 2000; Maynard-Smith and Haigh 1974; Powers et al. 1991; Ruiz-Pesini et al. 2004). However, the McDonald–Kreitman test showed no evidence of selection on the present *Cytb* gene of *G. glis*. It is possible that selection acted on another gene or genes more directly linked to hibernation or cold resistance, as was observed in *Ochotona* (Yang et al. 2008). If so, this selection could have led to a European postglacial expansion from only a small number of cold-resistant animals, thereby resulting in the observed mitochondrial homogeneity.

The extremely low genetic diversity in *G. glis* suggests that this species may be vulnerable to rapid climatic shifts, as is thought to be the case for many other endangered species, such as mustelids (Michaux et al. 2004), the Tasmanian devil (*Sarcophilus harrisii*—Jones et al. 2004), and the cheetah (*Acinonyx jubatus*—O'Brien et al. 1986). Future work examining other glirids for concordant patterns may reveal a link between hibernation behavior and population structure in this family, and these studies should have important implications for conservation of *G. glis* and other potentially threatened glirid species. However, before reliable conservation-related conclusions can be drawn from this study, more intensive sampling of *G. glis* in Italy and examination of additional genetic markers, such as microsatellites (currently being examined in our laboratory), must be undertaken.

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APPENDIX I

Specimens examined.—Locality numbers (from Fig. 1), localities, number of animals (*n*), haplotype designations, voucher numbers, and GenBank accession numbers for animals analyzed in this study. GenBank numbers are provided for unique haplotypes only, and haplotypes downloaded from GenBank are indicated by an asterisk (*). Collections of tissue samples: Estación Biológica de Doñana (Spain)—EBD 1265, 1136, 2992; University of Primorska (Slovenia)—GgCz 1, 2, GgTu 2–4, GgS 45–49, GgC 1–5, GgBH 1–7, GgI 5–8, GgMa 1, GgMo 1; Università di Palermo (Italy)—GgI 10, 11, 13, 14, 17–19, 23, 24, 28–32, 36–39, 41, 43, 44, GgLet 1–3; Universität Frankfurt (Germany)—GgA 7, 10, 12, 14–17. All other tissue samples are stored in the Botanic Institute of University of Liege.

Locality no.	Locality	*n*	Haplotype designation	Voucher no.	GenBank no.
Edible dormouse (*Glis glis*)					
1	Spain: Lugo	1	Hap02	EBD 1265	FM160652
2	Spain: Cantabria	1	Hap01	EBD 1136	FM160651
3	Spain: Navarra	1	Hap02	EBD 2992	
4	Spain: Arties	1	Hap02	GgE 50	
5	Spain: Montseny and Montnegre Natural Park (MNP)	9	Hap02, 03	GgE 1–3, 13, 14, 53–55	FM160652, FM160653
6	Spain: Gresolet (+StJulia)	4	Hap02	GgE 17, 29, 30, 32	
7	Spain: Vidra (+Grevulosa)	6	Hap02	GgE 18–20, 33–35	
8	United Kingdom: Tring	5	Hap02, 16	GgT 4–8	FM160652, FM160665
9	France: Py	1	Hap02	GgF 2	
10	France: St-Maxime	1	Hap05	GgF 18	FM160655
11	France: Montarnaud	4	Hap02	GgF 12, 17, 21, 23	
12	France: Vercors	2	Hap04	GgF 9, 10	FM160654
13	Belgium: Gaume	9	Hap01, 02	GgB 7, 8, 16, 22–24, 34, 35, 38	
14	Switzerland: Lausanne	1	Hap02	GgV 2	
15	Switzerland: Sundlauenen	1	Hap02	GgV 1	
16	Switzerland: Lago Maggiore	4	Hap02	GgV 3–6	
17	Germany: Grumst	2	Hap01	GgA 7, 10	
18	Germany: Bellings-Sterbfritz-Weinberg	5	Hap01	GgA 12, 14–17	
19	Italy: Torino-Viu	5	Hap02	GgI 7, 8, 28–30	
20	Italy: Druento	1	Hap02	GgI 5	
21	Italy: Asti	2	Hap02	GgI 6, 31	
22	Italy: Asiago	2	Hap02	GgI 10, 11	
23	Italy: Bologna	2	Hap06	GgI 13, 14	FM160656
24	Italy: San Fili	1	Hap09	GgI 19	FM160734
25	Italy: Aspromonte	5	Hap07, 08, 12	GgI 2–4, 17, 18	FM160657, FM160733, FM160660
26	Italy: Eolian Island (Salina)	2	Hap10	GgI 23, 24	FM160658
27	Sicily: Madonie	7	Hap07, 13, 14	GgI 36–39, 41, 43, 44	FM160657, FM160661, FM160662
28	Sardinia	1	Hap11	GgI 32	FM160659
29	Slovenia: Mt. Krim	5	Hap02	GgS 7–9, 29, 30	
30	Slovenia: Semič	3	Hap02	GgS 45–47	
31	Slovenia: Mt. Pohorje	2	Hap02	GgS 48, 49	
32	Croatia: Mt. Svilaja	5	Hap02	GgC 1–5	
33	Czech Republic: Mt. Jeseniki	2	Hap02	GgCz 1, 2	
34	Bosnia and Herzegovina: Mt. Zelengora	7	Hap02	GgBH 1–7	
35	Montenegro: Šavnik-Nikšić	1	Hap02	GgMo 1	
36	Macedonia: Mt. Galičica	1	Hap15	GgMa 1	FM160664
37	Hungary: Nanaly	7	Hap02	GgH 1, 3–8	
38	Latvia: Gaujas	1	Hap02	GgLet 2	
39	Latvia: Turaida	1	Hap02	GgLet 1	
40	Latvia: Skriveri	1	Hap02	GgLet 3	
41	Turkey: Istranca	2	Hap02	GgTu 2–4	
42	Turkey: Uludag	1	Hap02	GgTu 1	
43	Russia: Zhiguli Forest	5	Hap02	GgR 5, 10–13	
Hazel dormouse (*Muscardinus avellanarius*)—outgroup 1 (Bentz and Montgelard 1999)		1			AJ225117*
Garden dormouse (*Eliomys quercinus*)—outgroup 2 (Bentz and Montgelard 1999)		1			AJ225030*
		1			FM164278
Asian garden dormouse (*Eliomys melanurus*)—outgroup 3		2			FM164279, FM164280