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Using phylogeography to promote dormouse conservation: the case of *Muscardinus avellanarius* (Rodentia, Gliridae)

ALICE MOUTON, ANDREA GRILL, MAURIZIO SARÁ, BORIS KRYŠTUFEK, ETTORES RANDI, GIOVANNI AMORI, RIMVYDAS JUŠKAITIS, GAETANO ALOISE, ALESSIO MORTELLITI, GOEDELE VERBEYLEN, FABINA PANCHETTI, FRANCOISE POITEVIN & JOHAN MICHAUX

Abstract

This study describes the phylogeographic history of the common dormouse, *Muscardinus avellanarius*, a rodent strictly protected in Europe (Habitat Directive, annex IV; Bern Convention, annex III). We analyzed the genetics of 120 common dormice across the species' range, using sequences data from the mitochondrial cytochrome b gene (704 pb). The dataset obtained was analyzed using different phylogenetic reconstructions as well as other methods adapted to phylogeography. A complex phylogeographic pattern has been retrieved from the mitochondrial DNA gene, with the presence of two highly divergent lineages. These two lineages are themselves subdivided into five sublineages, which should be regarded as independent conservation units. Low genetic diversity was observed within each of the lineages, in contrast to an important level of genetic differentiation between them. These results have important implications for the conservation of this dormouse and will help to propose the best management measures for this species.

Keywords: Cytochrome b, glacial refugia, conservation genetic

1. Introduction

Sharp biological conservation of mammals or other terrestrial vertebrates in Europe needs detailed data on the phylogeography, genetic diversity, structure of present populations, as well as on the dynamics of past populations (Randi 2003). The detection of a phylogeographic structure is important as it helps in identifying long-term isolated populations that might have distinct gene pools and local adaptations, thus orienting concrete conservation actions aimed at avoiding outbreeding and gene pool disruption. Across the Paleartic, phylogeographic structures are generally associated with the Quaternary climatic oscillations, as they have played a major role in shaping the present geographical distribution of the species and their genetic diversity (Avise 2000). Populations were repeatedly isolated in different refugia, during glacial peaks, leading to various re-colonization patterns when the climate improved (Bilton et al. 1998, Taberlet et al. 1998, Schmitt 2007, Castiglia et al. 2009). Most of the studies have indicated that southern Europe, Asia Minor and the Caucasus acted as glacial refugia for taxa that are now widespread in Europe (Hewitt 2000), although some species also persisted in more northern refugia (Kotlik et al. 2006).

Gliridae are one of the most ancient rodent families, emerging in the Eocene (between 54–37 Mya) (Nadachoswki & Daoud 1995). Most of the 28 species contained in the family

Gliridae (Holden 2005) are now regarded as rare or endangered, attracting conservation-related research and active habitat management to support their survival (Morris 2003). So, the conservation concern for the common dormouse, *Muscardinus avellanarius*, makes it an excellent candidate for such a study. The common dormouse is strictly protected as it has declined in numbers and distribution in several countries (e.g. The Netherlands, Denmark and the UK) due to its complex biological requirements hampered by habitat deterioration and fragmentation (Foppen et al. 2002, Vilhelmsen 2003, Bright et al. 2006). A recent genetic study in England concluded that combination of localised breeding, high sites fidelity in females and short range dispersal in males could contribute to the formation of genetic structuring at a small spatial scale in common dormouse (Nam et al. 2011). However, despite the increasing interest for the common dormouse, we are far from having a good knowledge of the phylogeographic pattern of this species in Europe. The aim of this study is therefore to examine the phylogeography of the common dormouse in Europe.

2. Materials and methods

A total of 120 tissue samples from *M. avellanarius* spread throughout the species' range were analyzed (Fig. 1, Tab. 1). The specimens (road-killed and live trapped animals) were obtained by the authors and their field collaborators (see the Acknowledgements). Tissues and hairs were preserved in 96% ethanol until DNA extraction. An additional sequence from Switzerland was downloaded from the GenBank database (Bentz & Montgelard 1999).

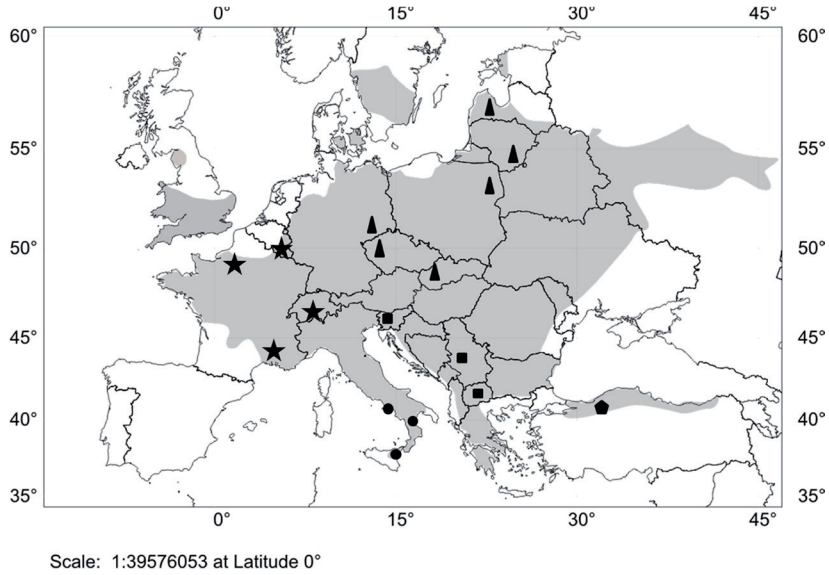

Fig. 1 Geographical distribution of the *Muscardinus avellanarius* in Europe and proximate location of the sampled populations. The shaded zone corresponds to the distribution area of the species. Different symbols refer to lineages in Fig. 2 and in the Tabs 2, 3 (★ = West European sub-lineage, ● = Italian sub-lineage, ⬟ = Turkish specimen, ▲ = Central North European sub-lineage, ■ = Balkans sub-lineage).

Tab. 1 Map references, geographic locations, corresponding sublineages, haplotypes and GenBank accession numbers of *Muscardinus avellanarius* haplotypes used in this study.

Geographic origin		Sub lineages	Total numbers of animals	Haplotypes	Genbank accession number
Hazel dormouse (*Muscardinus avellanarius*)					
Macedonia	Mt. Galičica	Balkans	5	Hap01, 04, 05	FN796753 FN796756 FN796757
	Popova Šapka	Balkans	1	Hap05	FN796757
	Mt. Pelister	Balkans	1	Hap05	
Serbia	Mt. Cer	Balkans	1	Hap05	
Slovenia	Mt Krim	Balkans	2	Hap03, 05	FN796755
	Pogorelec Mt. Kocevski Rog	Balkans	1	Hap02	FN796754
Lithuania	Šakiai district	Central North	16	Hap06, 07,08, 09	FN796758 FN796759 FN796760 FN796761
Latvia		Central North	3	Hap11, 12,13	FN796763 FN796764 FN796765
Czech Republic		Central North	2	Hap 16	FN796768
Slovakia		Central North	6	Hap 15, 36	FN796767 HE799313
Poland		Central North	2	Hap10, 15	FN796762 FN796767
Germany	Saxony	Central North	3	Hap06, 14,18	FN796758 FN796766 FN796770
Turkey	Mt.Ulu dag	Turkish	1	Hap17	FN796769
Switzerland	Canton de Vaud	West European	6	Hap19	FN796771
	(Bentz & Montgelard 1999)	West European	1	Hap22	AJ225117*
France	Normandie	West European	2	Hap20	FN796772
	South of France	West European	2	Hap 37	HE799314
Belgium	Mechelen	West European	16	Hap21	FN796773
Italy	Tevere Farfa (Lazio)	Italian	1	Hap23	FN796774
	Castel di Guido (Lazio)	Italian	13	Hap24, 27	FN796775 FN796776
	Arcinazzo Romano (Lazio)	Italian	1	Hap24	FN796775
	Viterbo (Lazio)	Italian	4	Hap24, 26, 28	FN796777 FN796779

Tab. 1 (see previos page)

Geographic origin		Sub lineages	Total numbers of animals	Haplotypes	Genbank accession number
	Filettino (Lazio)	Italian	1	Hap25	FN796776
	Castelporziano (Lazio)	Italian	12	Hap24, 27,28	
	Perugia (Umbria)	Italian	2	Hap24	
	Calabria	Italian	1	Hap29	FN796780
	Cosenza (Calabria)	Italian	1	Hap29	
	Catena Costiera (Calabria)	Italian	1	Hap29	
	High Madonia (Sicily)	Italian	4	Hap30, 32, 33	FN796781 FN796783 FN796784
	Low Madonia (Sicily)	Italian	2	Hap31, 32	FN796782 FN796783
OUTGROUPS					
Garden dormouse (*Eliomys quercinus*)					
	(Bentz & Montgelard 1999)		1		AJ225030*
	(Bentz & Montgelard 1999)		1		FM16427*
			1		FR848958
			1		FR848957
Asian Garden dormouse (*Eliomys melanurus*)					
			1		FR848955
			1		FR848956
Edible dormouse (*Glis glis*)					
	(Hürner et. al. 2010)		1		FM16065*

Total DNA was extracted using the DNeasy Tissue kit (Qiagen Inc.,Valencia, CA, USA) following the manufacturer's instructions. A fragment of 704 bp was sequenced from cytochrome b (cyt b) of the mitochondrial DNA gene (mtDNA). PCR amplifications were carried out using primers designed by Andrea Grill specifically for *M. avellanarius*, modified from Bentz & Montgelard (1999): LMA14255, 5′-TGGTGGAATTTCGGTTCTCT-3′; RMA15192, 5′-GTTGGCCTCCAATTCATGTT-3′. In order to recover the degraded material, two further internal specific primers were designed by fragment alignment: MUSCAR_ RINTERN, 5′-AAGGTGAACTATTACTAGGGC-3′, combined with LMA14255 and MUSCAR_LINTERN, 5'-ACCCTAGTAGAATGAATCTGA-3, combined with RMA15192. The DNA extraction and amplification methods are detailed in Mouton et al. (2012).

Phylogenetic trees were reconstructed for the phylogeographic purposes using two probabilistic approaches: the maximum likelihood method (ML) and Bayesian inferences (BI) as described in Mouton et al. (2012). A median joining network was performed with NETWORK v4.5.1.6. (Bandelt et al. 1999) to explore the relationships among the haplotypes of the dataset. Haplotype (h) and nucleotide (p) diversities (Nei 1987), and their standard

deviations (Tajima 1989) were calculated in DNASP v5 (Librado & Rozas 2009). The net distance between groups and average distances within groups were calculated in MEGA 4 (Tamura et al. 2007). Relative rate tests and an approximate time of divergence between the observed mtDNA lineages were calculated as detailed in Mouton et al. (2012).

3. Results

A total of 35 haplotypes was identified among the 120 samples. The phylogenetic tree (Fig. 2) and the Median Joining Network (data not shown) gave similar results and indicated that the haplotypes clustered into 2 lineages.

The first lineage (hereafter Lineage 1) split into two well-supported sublineages, the first of which encompassed individuals from Western Europe (Belgium, Switzerland, and France), whereas the second encompassed all the haplotypes from Italy. Lineage 2 includes populations

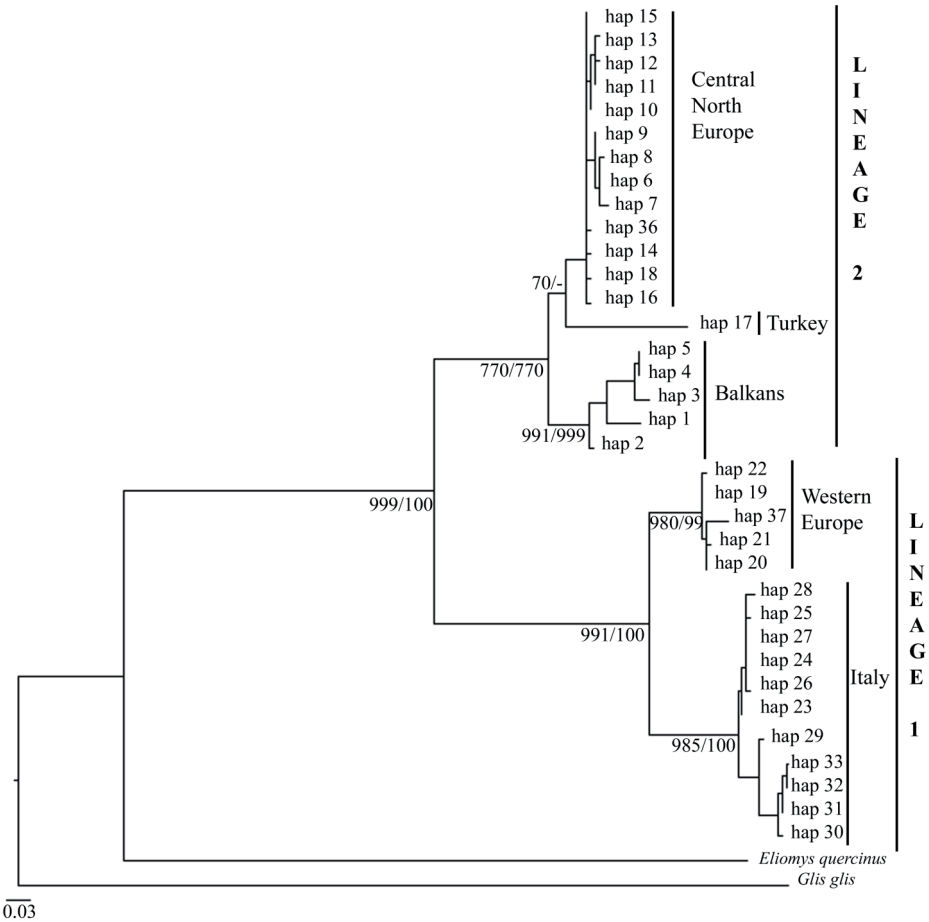

Fig. 2 Maximum-likelihood tree for the 35 haplotypes of *Muscardinus avellanarius*. Numbers indicated on the branches correspond to bootstrap support obtained in the ML analysis (left) and Bayesian probabilities (right). Haplotypes origins are indicated in Table 1.

from Central Northern Europe (Lithuania, Germany, Latvia, Czech Republic, Slovakia), from the Balkan Peninsula (Slovenia, Serbia, Macedonia) and Turkey. Further substructuring is poorly evident for this major second lineage. However, the Balkan sublineage is well supported and may hold a sister position to that of the population from Central North Europe.

The values of the haplotype and nucleotide diversities are summarized in Table 2 and generally indicate a very low level of diversity in both Lineage 1 and 2. The highest nucleotide diversities were found in the Balkan and the Italian sublineages, with average Pi values of 0.0067 and 0.0066 respectively.

Tab. 2 Summary of haplotypes (Hd) and nucleotide diversity (Pi), and their standard deviations, observed within the main genetic groups of *Muscardinus avellanarius*.

	Sample size (n)	**Pi ± SD**	**Hd ± SD**
All	120	0.055 ± 0.001	0.912 ± 0.013
Lineage 2	50	0.014 ± 0.002	0.0004 ± 0.046
Balkans	11	0.0067 ± 0.003	0.491 ± 0.175
Central North Europe	38	0.003 ± 0.0004	0.742 ± 0.079
Turkey	1	/	/
Lineage 1	70	0.025 ± 0.001	0.093 ± 0.028
Western Europe	31	0.002 ± 0.0005	0.553 ± 0.093
Italy	39	0.0066 ± 0.001	0.733 ± 0.001

The net genetic distance between Lineage 1 and Lineage 2 (9%) and among all the sublineages is very high (Tab. 3) and corresponds to a high differentiation among the lineages. The approximate time of divergence between Lineage 1 and Lineage 2 is around 10 Mya. The split between the Western and Italian sublineages and between the Balkan and the Central North sublineages should have taken place around 3.3 Mya.

Tab. 3 Percent of cytochrome b net genetic distance (NGD) between lineages.

	Sample size (n)	**Balkans** NGD	**Central North Europe** NGD	**Western Europe** NGD	**Italy** NGD
A 1	120	/	/	/	/
Lineage 2	50	/	/	/	/
Balkans	11	/	3	9.1	9.2
Central North Europe	38	3	/	/	9.2
Turkey	1	/	/	/	/
Lineage 1	70	/	/	/	/
Western Europe	31	/	9	/	/
Italy	39	/	/	3.4	/

4. Discussion

As already observed in a previous genetic study of the common dormouse (Mouton et al. 2012), our mtDNA study gives evidence for the presence of two highly divergent genetic lineages for *M. avellanarius* within Europe. Lineage 1 is spread within Western Europe and Italy and Lineage 2 in Central Europe, in the Balkan Peninsula and in Turkey. The genetic divergence (9%) between these two lineages is important and falls within the range of inter-specific cyt *b* distances observed in mammals in general, and in Glirinae in particular (divergence between the garden dormouse, *Eliomys quercinus* and the Asian garden dormouse, *E. melanurus* = 7.2%). Furthermore, these two lineages are subdivided into five sublineages that are genetically isolated, with a strong geographical association. According to our estimations of divergence time, Lineages 1 and 2 were separated 10 Mya, whereas the different sublineages diverged around 3.3 Mya. These dates correlate with important periods of climatic and faunal changes in Europe. Indeed, the Late Miocene (11 Mya - 5 Mya) has been an important period for the diversification of European mammals such as moles, *Talpa* spp. (Colangelo et al. 2010), European hedgehog, *Erinaceus europaeus* and the Northern White-breasted hedgehog, *Erinaceus roumanicus* (Santucci et al. 1998), and the eastern and western clade of the red deer *Cervus elaphus* (Ludt et al. 2004).

Later, the important climatic oscillations characterizing the Late Pliocene and the Quaternary led to the appearance of the five different *M. avellanarius* sublineages.

The high level of genetic diversity in the Balkan Peninsula, and in Italy, strongly suggests that these regions were refugia for *M. avellanarius* during the Quaternary glaciations. However, the high level of endemism of the Balkan and Italian sublineages would suggest that these populations did not contribute to postglacial recolonization of Central Northern Europe and Western Europe, respectively. Our results rather suggest that the modern populations of *M. avellanarius* from Western Europe and Central Northern Europe might derive from populations that surviving outside the traditionally accepted refugia, namely the Carpathian region as occuring for several other species (Seddon et al. 2002, Deffontaine et al. 2005, Kotlik et al. 2006). However, this hypothesis waits to be confirmed by a more extensive sampling in the Balkan Peninsula, in Eastern and Central Northern Europe.

The high genetic divergences observed among all the lineages and sublineages, as well as the estimation of the ancient divergence times among them, tend to suggest a low dispersal of the common dormouse population across Europe. However, this hypothesis has to be confirmed by additional molecular markers and a more complete sampling. Finally, a recent phylogeographic study on the edible dormouse (*Glis glis*) (Hürner et al. 2010) revealed a totally different phylogeographic pattern. Indeed, this species is characterized by great genetic homogeneity in Europe and very low genetic diversity in the Mediterranean Peninsulas. The study of the phylogeographic structure of the garden dormouse is in progress by our team and seems to give another original pattern. A phylogeographic study on the forest dormouse (*Dryomys nitedula*) would be also interesting in order to compare its genetic structures with the other European Gliridae. However, no genetic information is available for this species.

The use of comparative phylogeographic approaches, will allow us to better understand why these closely related species seem to have reacted so differently to past environmental changes. These results will also help to propose adequate conservation measures for these rare and often endangered species.

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Authors' addresses:

Alice Mouton*, Johan Michaux
Institut de Botanique, bât. 22
Université de Liège (Sart Tilman)
Boulevard du Rectorat 27
4000 Liège, Belgium

Andrea Grill
Institut Biodiversität der Tiere
Universität Wien
Rennweg 14
1030 Vienna, Austria

Maurizio Sará
Dipartimento Biologia Animale
Laboratorio di Zoogeografia ed Ecologia Animale
Via Archirafi
90123 Palermo, Italy

Boris Kryštufek
Science and Research Centre
University of Primorska
Garibaldijeva 1
SI-5000 Koper, Slovenia

Ettores Randi
ISPRA ex INFS
Ozzano Emilia
40064 Bologna, Italy

Giovanni Amori
CNR
Institute of Ecosystem Studies
Via A. Borelli 50
00161 Rome, Italy

Rimvydas Juškaitis
Institute of Ecology of Nature Research Centre,
Akademijos 2
LT-08412 Vilnius, Lithuania

Gaetano Aloise
Museo di Storia Naturale della Calabria e Orto Botanico
Via P. Bucci s.n.
87036 Rende (Cosenza), Italy

Alessio Mortelliti
Dipartimento di Biologia Animale e dell'Uomo
Università 'La Sapienza'
Viale dell'Università 32,
00185 Roma, Italy

Goedele Verbeylen
Natuurpunt Studie / Mammal Working Group
Coxiestraat 11
2800 Mechelen, Belgium

Fabina Panchetti
Dipartimento di Biologia
Università "Roma Tre"
Viale Guglielmo Marconi 446
00146 Roma, Italy

Francoise Poitevin
Centre d'écologie Fonctionnelle et évolutive
UMR 5175, CNRS
Route de Mende 34293
34967 Montpellier, France

*Corresponding author: Mouton Alice (email: amouton@ulg.ac.be)