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Genetic variation and evolution in the genus *Apodemus* (Muridae: Rodentia)

MARIA GRAZIA FILIPPUCCI[1], MILOŠ MACHOLÁN[2]* and JOHAN R. MICHAUX[3]

[1]*Department of Biology, University of Rome 'Tor Vergata', Via della Ricerca Scientifica, I-00133 Rome, Italy*
[2]*Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Veveří 97, CZ-60200 Brno, Czech Republic*
[3]*Laboratory of Palaeontology, Institut des Sciences de l'Evolution de Montpellier (UMR 5554), University of Montpellier II, Place E. Bataillon, F-34095 Montpellier Cedex 05, France*

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Genetic variation was studied using protein electrophoresis of 28–38 gene loci in 1347 specimens of *Apodemus agrarius*, *A. peninsulae*, *A. flavicollis*, *A. sylvaticus*, *A. alpicola*, *A. uralensis*, *A.* cf. *hyrcanicus*, *A. hermonensis*, *A. m. mystacinus* and *A. m. epimelas*, representing 121 populations from Europe, the Middle East, and North Africa. Mean values of heterozygosity per locus for each species ranged from 0.02 to 0.04. Mean values of Nei's genetic distance (*D*) between the taxa ranged from 0.06 (between *A. flavicollis* and *A. alpicola*) to 1.34 (between *A. uralensis* and *A. agrarius*). The highest values of *D* were found between *A. agrarius* and other *Apodemus* species (0.62–1.34). These values correspond to those generally observed between genera in small mammals. Our data show that *A. agrarius* and *A. peninsulae* are sister species, well-differentiated from other taxa. High genetic distance between *A. m. mystacinus* and *A. m. epimelas* leads us to consider them distinct species and sister taxa to other Western Palaearctic species of the subgenus *Sylvaemus*. The data also suggest a recent separation of members of the latter group from a common ancestor, and subsequent rapid radiation, making it difficult to infer phylogenetic relationships. Some taxonomic implications of the results are discussed further. *Biological Journal of the Linnean Society*, 2002, **75**, 395–419.

ADDITIONAL KEYWORDS: electrophoresis – genetic differentiation – Palaearctic region – phylogeny – *Sylvaemus* – wood mice.

INTRODUCTION

Wood mice of the genus *Apodemus* Kaup, 1829 are widespread in temperate areas of the Palaearctic region. Traditionally, the genus has been subdivided according to Zimmermann (1962) into three subgenera: *Apodemus*, known from central Europe to eastern Asia, western Palaearctic *Sylvaemus*, and Eastern Palaearctic *Alsomys* (see Musser & Carleton, 1993 for a general review). Recently, however, Musser *et al*. (1996), focusing on the *Apodemus–Sylvaemus* systematic problem, have separated wood mice into three groups: *Sylvaemus* (*A. sylvaticus*, *A. flavicollis*, *A. uralensis*, *A. mystacinus*, *A. fulvipectus*, *A. hermonensis*, *A. alpicola*, *A. arianus*, *A. hyrcanicus*, *A. ponticus*, *A. rusiges*, *A. wardi*), *Apodemus* (*A. agrarius*, *A. chevrieri*, *A. speciosus*, *A. peninsulae*, *A. latronum*, *A. draco*, *A. semotus*, *A. gurkha*), and *Argenteus* (*A. argenteus*). Serizawa *et al.* (2000), on the basis of DNA sequences of nuclear (the first exon of the *IRBP* gene) as well as mitochondrial (cytochrome b) markers, confirmed this classification, but replaced the term '*Sylvaemus* group' with '*Sylvaticus* group' and introduced a fourth group for the Asiatic species *A. gurkha* ('*Gurkha* group').

In the last three decades, the attention of zoologists has mainly been focused on the European species, *A. sylvaticus* Linnaeus 1758, and *A. flavicollis* Melchior 1834, characterized by widely overlapping ranges and

*Corresponding author. E-mail address: macholan@iach.cz

by morphological convergence in the southern parts of their ranges. According to several authors (Engländer & Amtmann, 1963; Witte, 1964; Amtmann, 1965), this convergence is the consequence of introgressive hybridization. However, this possibility was excluded by Niethammer (1969) on morphological evidence, and by numerous authors after allozyme studies (Engel *et al*., 1973; Debrot & Mermod, 1977; Benmehdi *et al.*, 1980; Csaikl *et al.*, 1980; Nascetti *et al.*, 1980; Gemmeke & Niethammer, 1981; Fraguedakis-Tsolis *et al*., 1983; Nascetti & Filippucci, 1984; Gebczyński *et al*., 1986), mtDNA restriction and species-specific PCR amplification patterns (Michaux *et al*., 1998a,b, 2001; Libois *et al*., 2001), and sequence studies (Chelomina *et al*., 1998; Serizawa *et al*., 2000; Suzuki, Tsuchiya & Takezaki, 2000).

In Europe, the subgenus *Sylvaemus* is represented by five species: *A. flavicollis*, *A. sylvaticus*, *A. alpicola* Heinrich 1952, *A. microps* Kratochvíl & Rosický, 1952, and *A. mystacinus* Danford & Alston 1877 (Fig. 1). *Apodemus alpicola*, originally described as a high altitude subspecies of *A. flavicollis*, was shown to be a morphologically (Storch & Lütt, 1989) and genetically (Vogel *et al*., 1991; Filippucci, 1992) well-defined species. The taxon *microps* has recently been synonymized with *A. uralensis* (Pallas 1811) (Vorontsov *et al*., 1992; Filippucci *et al.*, 1996; Mezhzherin, 1996; Bellinvia *et al*., 1999; Macholán *et al*., 2001a).

Apodemus mystacinus, occurring on the Balkan Peninsula and the Middle East, is clearly morphologically distinguishable from other *Sylvaemus* species and therefore was separated into the subgenus *Karstomys* Martino 1939 by some authors (Rietschel & Storch, 1974; Storch, 1975). However, the validity of *Karstomys* has not been generally accepted (Corbet, 1978; Niethammer, 1978; Musser *et al*., 1996). According to Felten *et al.* (1973), two subspecies should be recognized for the species: *epimelas*, distributed on the Balkan Peninsula, and nominal *mystacinus* of the Middle East (Fig. 1). Storch (1977) hypothesized a specific status for the two taxa, based on differences in the first upper molar found in the Recent as well as in Pleistocene populations. No direct genetic comparison between populations of *A. mystacinus* from different geographical areas has hitherto been carried out.

In North Africa only *A. sylvaticus* is present, in the Maghreb region. Saint Girons & Van Bree, 1963), investigating morphological traits, suggested three different subspecies to be present in North Africa: *A. s. hayi* (Waterhouse, 1837), inhabiting the Mediterranean regions of the Maghreb; *A.* s. *rufescens* Saint Girons & van Bree, 1963, inhabiting the Hauts Plateau in Algeria and arid forests in Morocco; and *A.* s. *ifranensis*, which has a range covering the Middle Atlas from the east of Khénifra to the region of Oulmes and to Ifrane (Saint Girons & van Bree, 1963; Saint

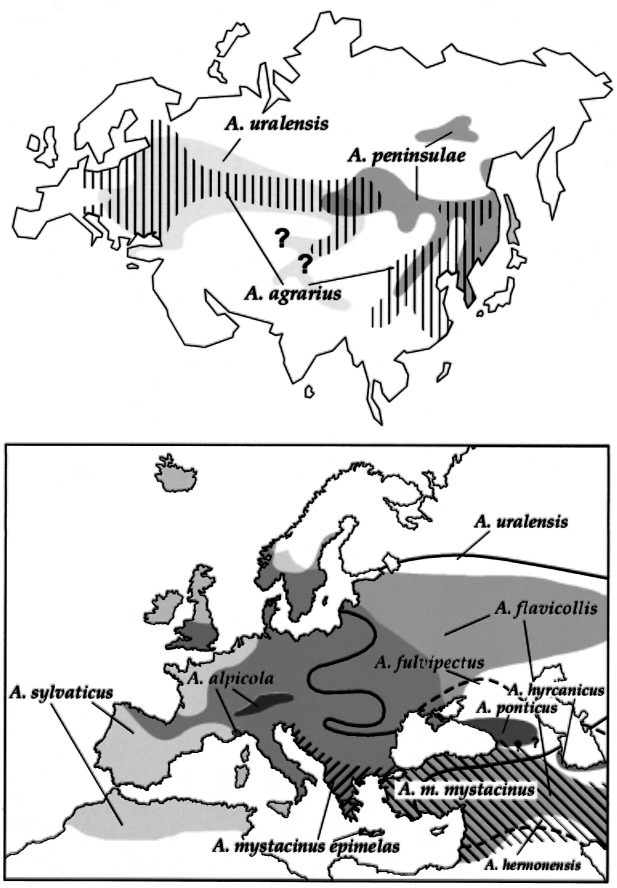

Figure 1. A schematic depiction of the geographical distribution of (top) *A. uralensis*, *A. peninsulae* and *A. agrarius* in Eurasia; and (bottom) *A. flavicollis*, *A. sylvaticus*, *A. alpicola*, *A. ponticus*, *A. fulvipectus*, *A. hermonensis*, *A. uralensis*, *A. hyrcanicus*, *A. mystacinus epimelas* and *A. m. mystacinus* in Europe and the Middle East, *A. uralensis* is indicated with a solid line *A. fulvipectus* and *A. hermonensis* (both indicated with a dashed line) are most probably conspecific. Compiled from Corbet (1978), Musser & Carleton (1993), Mezhzherin (1997b), Zagorodnyuk *et al*. (1997), Mitchell-Jones *et al*. (1999), and Macholán *et al*. (2001a).

Girons, 1972). However, Kock & Felten (1979) found no differences between *A. s. rufescens* and *A.* s. *hayi* and according to Kowalski & Rzebik-Kowalska (1991) and to Filippucci (1992), there are no differences among the North African populations of *A. sylvaticus*. Libois *et al*. (2001), on the basis of mtDNA RFLPs, did not find any genetic difference between the three North African subspecies and suggested combining *A. s. rufescens* and *A.* s. *ifranensis* within *A. s. hayi*.

Recently, zoologists have focused on south-eastern Europe and the Middle East, where several taxa, previously assigned to *A. sylvaticus*, have been recognized as distinct species. In Israel, a new species, *A. hermonensis* Filippucci, Simson & Nevo, 1989, was identified

by biometric and protein electrophoretic analyses. Filippucci *et al.* (1989) found *A. flavicollis* (apart from *A. mystacinus*) to be the most common species in Israel, instead of *A. sylvaticus* as then commonly believed.

Several subspecies of *A. sylvaticus* (*uralensis* of the southern Ural, *charkovensis* of Ukraine, *mosquensis* of the Moscow region, *ciscaucasicus* of the northern Caucasus, and *tscherga* of the Altai) appeared to be geographical forms of another species, *A. uralensis*, according to allozyme data (Mezhzherin & Mikhailenko, 1991). (Recently, the taxa *mosquensis* and *ciscaucasicus* have been asserted to deserve the specific status within the superspecies *A. uralensis* by Orlov *et al*. (1996), mostly on the basis of cytogenetic studies.) A new species was also described from Ukraine, originally named *A. falzfeini* (Mezhzherin & Zagorodnyuk, 1989), and later synonymized with *A. fulvipectus* Ognev, 1924 from the Caucasus (Vorontsov *et al*., 1992). Vorontsov *et al*. (1992) used morphological, chromosomal and allozyme data to investigate the systematics of the *Sylavemus* group in the Caucasus and Transcaucasus. They proposed that four species inhabited those regions (Fig. 1): *A. ponticus* Sviridenko 1936, previously considered subspecies of *A. flavicollis* (Mezhzherin, 1991), *A. hyrcanicus* Vorontsov, Boyeskorov & Mezhezherin 1992, *A. uralensis*, and *A. fulvipectus* Mezhzherin, Boyeskorov & Vorontsov 1992 (Vorontsov *et al*., 1992).

In western Anatolia, four *Sylvaemus* species were suggested by electrophoretic and morphological analyses (Filippucci *et al*., 1996). *Apodemus sylvaticus*, previously considered widely distributed in Asian Turkey, was shown to be an extremely rare species there, restricted to a small area near the coast of the Black Sea. On the contrary, *A. flavicollis*, previously considered to be from the Caucasus and eastern Anatolia only, appeared to be widely distributed throughout the area studied, as also did *A. hermonensis*, previously known only from Mt. Hermon in Israel. Finally, *A. uralensis* was found to be confined to the humid mountainous areas of northern Asia Minor. These results were subsequently confirmed by Macholán *et al*. (2001a) who extended the study to eastern Turkey, Armenia, and to the western, northern and southern parts of Iran.

Nevertheless, our knowledge of the systematics of wood mice from Iran is still incomplete and the data from eastern parts of Iran and Nepal as well are rather fragmentary. Being previously attributed to *A. sylvaticus* according to external morphological characters, populations from this area displayed a higher genetic affinity to *A. flavicollis* than to *A. sylvaticus*, although differentiated from both of them (Darviche *et al*., 1979; Gemmeke & Niethammer, 1982). Recently, Musser & Carleton (1993), in their synopsis of mammal species of the world, suggested *A. arianus* (Blanford 1881) inhabited Iran, and *A. wardi* (Wroughton 1908) inhabited Nepal, Kashmir, Pakistan, Afghanistan, and north-western Iran. According to these authors, *A. fulvipectus* (in the north), and *A. ponticus* (in the north-west) probably also occur in Iran. More recently, Mezhzherin (1997b) and Zagorodnyuk *et al.* (1997) have suggested *A. arianus* to be an older synonym of the taxa *falzfeini*, *chorassanicus*, *fulvipectus*, and *hermonensis*.

Genetic differentiation and/or phylogenetic relationships among species of the genus *Apodemus* have been studied by many authors using both biochemical and molecular methods (see Michaux *et al*., 2002 and references therein). Because of the high genetic differentiation between *A. agrarius* and all other species from the western Palaearctic region, several authors (Bonhomme *et al*., 1985; Britton-Davidian *et al*., 1991; Filippucci, 1992; Filippucci *et al*., 1996; Mezhzherin, 1997b) have proposed *Sylvaemus* to be a distinct genus. Hartl *et al*. (1992), studying allozyme differentiation at 36 loci in four *Apodemus* species (*A. sylvaticus*, *A. flavicollis*, *A. microps*, and *A. agrarius*), *Mus*, and *Rattus*, and using *Microtus*, *Clethrionomys*, and *Cricetus* as outgroups, found *A. agrarius* to be more distant to other *Apodemus* species than were *Mus* and *Rattus* on a UPGMA phenogram, however, cladistic analysis of the same data showed monophyly of all the *Apodemus* taxa. Hartl *et al*. (1992) concluded that the results of electrophoretic studies had been greatly biased because of the unequal rates of genic evolution among the taxa and suggested using a cladistic approach for the inference of phylogenetic relationships within the genus. More recently, monophyly of the genus *Apodemus* has been corroborated by molecular studies (Chelomina *et al*., 1998; Serizawa *et al*., 2000; Michaux *et al*., 2002). Musser *et al*. (1996) recommended retention of the generic name *Apodemus* pending the systematic revision of the entire complex of species.

Notwithstanding conflicting results of different studies regarding phylogenetic relationships both within and among subgenera of the genus *Apodemus*, recent morphological, biochemical and molecular analyses seem to agree in that there are 3–4 phylogenetic lineages within the genus (*Sylvaemus*/*Sylvaticus*, *Apodemus*, *Argenteus*, *Gurkha*), however, elevating these lineages to separate genera is not substantiated. Although *A. mystacinus* and all *Sylvaemus* species most probably form a monophyletic group, relationships within the latter group remain unclear and also evidence supporting inclusion of *A. mystacinus* within a separate genus *Karstomys* (Martin *et al*. 2000) is inconclusive (Michaux *et al*., 2002).

The purpose of the present study is to extend the analysis carried out by Filippucci (1992) on allozyme

variation at 28–33 loci in 615 specimens representing 51 populations of seven *Apodemus* species (*A. sylvaticus*, *A. flavicollis*, *A. alpicola*, *A. microps* [*uralensis*], *A. hermonensis*, *A. mystacinus*, and *A. agrarius*), increasing the number of taxa and more than doubling the number of specimens and populations investigated. Data are presented on allozyme variation at 28–38 loci in the following taxa: *A. sylvaticus*, *A. flavicollis*, *A. alpicola*, *A. hermonensis*, *A. uralensis*, *A. mystacinus epimelas*, *A. m. mystacinus*, *A. peninsulae*, *A. agrarius*, and an unknown taxon from northern Iran we provisionally call *A.* cf. *hyrcanicus* (see Macholán *et al*., 2001a for details). Genetic differentiation and the phylogenetic relationships within and among species of the genus *Apodemus* are discussed.

MATERIAL AND METHODS

Electrophoretic analysis was carried out on 1347 specimens representing 121 populations of ten taxa of *Apodemus* from Europe, the Middle East and North Africa. Collecting sites, their abbreviations, and numbers of specimens examined for each population are presented in Appendix 1.

Tissues of each specimen were preserved at –80°C until processed. Homogenates for electrophoresis were obtained from portions of muscle or kidney tissue crushed in distilled water. Genic variation was assessed using standard horizontal gel electrophoresis. Homogenates obtained from muscle were processed for the following enzymatic systems: α-glycerophosphate dehydrogenase (E.C. 1.1.1.8; *αGpdh*), sorbitol dehydrogenase (E.C. 1.1.1.14; *Sdh*). lactate dehydrogenase (E.C. 1.1.1.27; *Ldh-1*, *Ldh-2*), malate dehydrogenase (E.C. 1.1.1.37; *Mdh-1*, *Mdh-2*), malic enzyme (E.C. 1.1.1.40; *Me-1*, *Me-2*), isocitrate dehydrogenase (E.C. 1.1.1.42; *Idh-1*, *Idh-2*), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44; *6-Pgdh*), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49; *G6pdh*), glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12; *G3pdh*), indophenol oxidase (E.C. 1.15.1.1; *Ipo-*, *Ipo-2*), nucleoside phosphorylase (E.C. 2.4.2.1; *Np*), glutamate-oxalacetate transaminase (E.C. 2.6.2.1; *Got-1*, *Got-2*), hexokinase (E.C. 2.7.1.1; *Hk-1*, *Hk-2*), creatine kinase (E.C. 2.7.3.2; *Ck*), adenylate kinase (E.C. 2.7.4.3; *Adk*), phosphoglucomutase (E.C. 2.5.7.1; *Pgm-1*, *Pgm-2*), esterases (E.C. 3.1.1.1; *Est-1*, *Est-2*, *Est-3*), leucyl aminopeptidase (E.C. 3.4.11; *Lap*), peptidases (E.C. 3.4.11; *Pep-1*, *Pep-2*, *Pep-3*), acid phosphatase (E.C. 3.1.3.2; *Acph*), adenosine deaminase (E.C. 3.5.4.4; *Ada*), aldolase (E.C. 4.1.2.13; *Aldo*), fumarase (E.C. 4.2.1.2; *Fum*), mannose phosphate isomerase (E.C. 5.3.1.8; *Mpi*), and glucose phosphate isomerase (E.C. 5.3.1.9; *Gpi*). Homogenates obtained from kidney were processed for alcohol dehydrogenase (E.C. 1.1.1.1; *Adh*). The electrophoretic procedures used were those described by Filippucci *et al*., 1988).

Isozymes were numbered in order of decreasing mobility from the most anodal one. Allozymes were numbered according to their mobility, relative to the most common allele (designed *100*) in the reference population of *A. sylvaticus* from Burano (SBUR). Allozyme data were analysed using allele frequencies as input. Intrapopulation genetic variation was estimated as the mean number of alleles per locus (A), proportion of polymorphic loci in the population ($P_{1\%}$), mean observed heterozygosity per locus (H_o), and mean expected heterozygosity per locus (genetic diversity, H_e; Nei, 1978). Genetic structure within and among conspecific populations was estimated by means of F-statistics (Wright, 1965). The amount of genetic divergence between populations was estimated with the indices of Nei's standard and unbiased genetic distance (Nei, 1972, 1978) and with chord distance introduced by Cavalli-Sforza & Edwards (1967; see Macholán *et al*., 2001b for the rationale behind using these indices; see also Swofford *et al*., 1996 for statistical details). BIOSYS-1 (Swofford & Selander, 1981) was used for all these procedures. Matrices of Nei's (1978) genetic distances were compared to geographical distances with the NTSYS-pc program (Rohlf, 1997) by comparing the observed with 'random' values of the Mantel Z statistic as obtained from 5000 permutations (Mantel, 1967). Matrices of geographical distances (rounded to 5 km, seas bypassed) were created using Microsoft© Encarta© World Atlas (1998 edition).

In total, 38 gene loci were studied, however, indices of genetic variation and distance were calculated on a lower number of loci, because for some of the species data were missing at a few loci. The present study includes new as well as previously reported data (Nascetti & Filippucci, 1984; Filippucci *et al*., 1989; Filippucci, 1992; Filippucci *et al*., 1996; Michaux *et al*., 1996a; Macholán *et al*., 2001a). Interspecific divergence was calculated on 34 shared loci, whereas intraspecific differentiation was evaluated on 28 loci in *A. flavicollis*, 29 loci in *A. sylvaticus*, 32 loci in *A. mystacinus*, 34 loci in *A. alpicola*, *A. agrarius* and *A. peninsulae*, and 36 loci in *A. hermonensis*, *A. uralensis* and *A.* cf. *hyrcanicus*.

Two loci (*Adh* and *Acph*) studied in Filippucci (1992) and Filippucci *et al*. (1996) were not included in the evaluation of genetic distances, because for some taxa (*A. peninsulae*, *A.* cf. *hyrcanicus*, and *A. m. epimelas*) data at these loci were missing. However, four new loci (*Est-1*, *Est-2*, *Pep-1*, and *Pep-2*) not included in Filippucci (1992) were considered for the analysis of interspecific differentiation.

Dendrograms of genetic relationships among populations within individual species were obtained using

the neighbour-joining method (Saitou & Nei, 1987) based both on Nei's (1978) and Cavalli-Sforza & Edwards' (1967) distances. In order to reveal phylogenetic relationships among the *Apodemus* species studied, we employed four different methods of phylogenetic inference which are based on different assumptions: maximum-likelihood (Felsenstein, 1981); Fitch–Margoliash procedure; Wagner parsimony based on mutation coding scheme proposed by Murphy (1993) and Murphy & Doyle (1998); and a method of constructing phylogenetic trees by applying the criterion of parsimony directly to allele frequency data as implemented in the program FREQPARS (Swofford & Berlocher, 1987). (This program tries to find the tree on which the frequency of each allele undergoes the least possible amount of change, while ensuring that allele frequencies in hypothetical ancestors add to one.)

Nei's (1972) standard distance was used for distance-based phylogenetic methods since (unlike Cavalli-Sforza & Edwards' procedure) it takes into account new mutations, more likely an explanation than mere drift for allelic differences between wood mouse species. In order to estimate reliability of phylogenetic inference at each node, the bootstrap method of Felsenstein (1985) with 1000 pseudoreplications was used for all phylogenies except those estimated by FREQPARS. The PHYLIP program package (Felsenstein, 1995) was used for all the procedures except when stated otherwise.

RESULTS

Pattern of variation

Overall, 190 alleles were scored. Thirty-five of them were rare and present with a frequency lower than 1% in at least one species. The highest number of alleles were found at *Ada* (12), *αGpdh* (11), and *Me-1* (11). Only a single locus, *Lap*, out of the 38 analysed loci was monomorphic and fixed for the same allele in all species examined. Three other loci showed alleles with frequencies lower than 1% in individual species: *Mdh-2* (with the allele *Mdh-2*^{92} present with 2% frequency only in the population of *A. sylvaticus* from the Lepini Mountains), *Ck* (with two rare alleles, *105* and *95*, present only in Israeli populations of *A. m. mystacinus*), *Adk* (with two rare alleles, *90* and *94*, present in populations of *A. sylvaticus* from the Lepini Mountains and Corsica).

Allele frequencies of the polymorphic and/or discriminant loci in the species analysed are given in Appendix 2. Only alleles with a frequency of at least 1% within all species were considered for interspecific comparisons. In Table 1, total numbers of alleles observed in each species are given, as well as the number of alleles in common and loci discriminating between pairs of species. *Apodemus flavicollis* displayed the highest number of alleles in common with other *Sylvaemus* species, ranging from 34 (with *A.* cf. *hyrcanicus*) to 46 (with *A. sylvaticus*). In contrast, *A. agrarius* displayed the lowest number of alleles in

Table 1. (A) Total number of loci studied, total number of alleles observed, and number of exclusive alleles with frequency higher or lower than 1% in each species. (B) Number of alleles in common (above diagonal) and number of discriminant loci (below diagonal) between species

	SYL	FLA	ALP	HER	URA	HYR	MYS	EPI	PEN	AGR
(A)										
No. loci	38	38	38	38	38	38	38	35	34	37
No. alleles	81	76	48	62	66	40	71	36	37	45
No. excl.alleles > 1%	9	2	–	4	2	2	12	6	4	15
No. excl.alleles < 1%	16	7	–	4	2	–	3	–	–	–
(B)										
sylvaticus	–	46	37	37	40	27	26	18	14	15
flavicollis	6	–	45	43	45	34	33	21	18	15
alpicola	5	1	–	37	39	33	27	17	12	11
hermonensis	6	3	4	–	40	30	27	16	14	15
uralensis	7	2	1	4	–	34	32	19	14	11
cf. *hyrcanicus*	7	4	4	6	3	–	28	18	15	11
mystacinus	8	10	11	13	9	10	–	27	16	14
epimelas	11	15	18	18	16	17	8	–	13	12
peninsulae	20	20	23	21	21	22	19	21	–	19
agrarius	21	22	26	22	26	24	23	22	16	–

common with other *Apodemus* species, ranging from 11 (with *A. alpicola*, *A. uralensis*, and *A.* cf. *hyrcanicus*) to 19 (with *A. peninsulae*).

A. sylvaticus

Eighty-one alleles were observed at 38 loci, 25 of them being exclusive to this species (Table 1). Intraspecific analysis was carried out on 29 loci. Twenty loci were polymorphic in the 42 populations analysed, whereas nine loci were monomorphic (*Sdh*, *Idh-2*, *G6pdh*, *G3pdh*, *Ipo-2*, *Hk-1*, *Ck*, *Acph*, *Lap*). Additional loci were included into interspecific comparisons: two of them (*Adh*, *Pgm-1*) were polymorphic and seven (*Hk-2*, *Est-1*, *Est-2*, *Pep-1*, *Pep-2*, *Pep-3*, *Acph*) were monomorphic. One exclusive allele was found in North Africa ($\alpha Gpdh^{104}$), whereas $Me\text{-}2^{115}$ was found in Algeria and the Iberian Peninsula. Other alleles exclusive to Spain were found at *Mdh-1* (*110*) and at *Me-2* (*92*). Several alleles at numerous loci were exclusive to populations from peninsular Italy: $Mdh\text{-}2^{92}$, $Me\text{-}2^{94}$, $Me\text{-}2^{88}$, $6Pgdh^{92}$, $Ipo\text{-}1^{82}$, $Got\text{-}1^{90}$ (found also in Sardinia), Adk^{94}, $Pgm\text{-}2^{96}$, Ada^{90}, Ada^{85}, Ada^{80}, and $Aldo^{105}$. Concerning insular populations, Corsica was characterized by four exclusive alleles $Ldh\text{-}2^{92}$, Np^{95}, Adk^{90}, and Fum^{96}. At a fifth locus, an allele $Got\text{-}2^{92}$ was exclusive to Corsica and Elba. The Sardinian population was characterized by the presence of an exclusive allele $Got\text{-}1^{90}$, observed in the mainland population from Penne. Populations from the Balkan Peninsula were characterized by the alleles $Got\text{-}1^{110}$ and $Ipo\text{-}1^{110}$.

A. flavicollis

Seventy-six alleles were observed at 38 loci and nine of them were exclusive to this species (Table 1). Intraspecific analysis was carried out on 28 loci. Fourteen loci were polymorphic in 30 populations, whereas 14 loci were monomorphic (*Sdh*, *Ldh-2*, *Mdh-2*, *Idh-2*, *Ipo-1*, *Ipo-2*, *Np*, *G6pdh*, *Got-2*, *Ck*, *Adk*, *Lap*, *Aldo*, *Fum*). Another five monomorphic loci (*Adh*, *Hk-2*, *Est-1*, *Acph*, *Pep-2*) and five polymorphic loci (*Me-1*, *Pgm-1*, *Est-2*, *Pep-1*, *Pep-3*) were added for interspecific comparisons. Several loci showed alleles exclusive to peninsular Italy: $\alpha Gpdh^{104}$, $Ldh\text{-}1^{92}$, $Mdh\text{-}1^{90}$, $Idh\text{-}1^{90}$, Adk^{94}, $Pgm\text{-}2^{96}$, $Est\text{-}3^{107}$, Ada^{85}, and Mpi^{95}. Two loci, *6Pgdh* and *Est-3*, contributed to a partial discrimination of peninsular populations from those sampled in Europe and the Middle East: whereas the allele $6Pgdh^{92}$ reached a mean frequency of 0.68 on the peninsula, it was only 0.19 in other populations. Likewise, the frequency of $Est\text{-}3^{95}$ was as high as 0.99 in peninsular populations, whereas in other parts of Europe the frequency was much lower (0.25) and in the Middle East the allele was absent and replaced by $Est\text{-}3^{105}$. Alleles $\alpha Gpdh^{106}$ and $Hk\text{-}1^{104}$ were present only in populations from Israel. At several loci, numerous alleles were found only in European populations: Ada^{95}, Pgi^{90} (the Balkans and Sweden), Pgi^{104} (Germany), $Me\text{-}2^{80}$ (Sweden), and $Ldh\text{-}1^{108}$ (the Alpine populations from Tarvisio and Vorarlberg). $Idh\text{-}1^{100}$, typical of *A. sylvaticus*, was found occasionally in European populations of *A. flavicollis*.

A. hermonensis

In total, 62 alleles were observed at 38 loci, eight of them being species-specific (Table 1). Intraspecific analysis was carried out on 36 loci in 20 populations; 19 loci were monomorphic (*Sdh*, *Ldh-1*, *Mdh-1*, *Mdh-2*, *Idh-2*, *G6pdh*, *G3pdh*, *Ipo-2*, *Np*, *Got-2*, *Hk-1*, *Hk-2*, *Adk*, *Pgm-2*, *Ap-3*, *Lap*, *Aldo*, *Fum*, *Mpi*). Two additional loci were considered for interspecific comparisons: *Acph* (monomorphic), and *Adh* (polymorphic). Three alleles were exclusive to Iranian populations: $Ldh\text{-}2^{97}$, $Idh\text{-}1^{113}$, and Ck^{95}. Two alleles were characteristic of Israeli populations: $Est\text{-}3^{95}$ and Ada^{115}, and Turkish populations were characterized by $\alpha Gpdh^{93}$, $6Pgdh^{112}$, $Ipo\text{-}1^{70}$, $Pgm\text{-}1^{95}$, $Ap\text{-}2^{90}$, $Est\text{-}3^{103}$, and Pgi^{104}.

A. alpicola

Of 48 alleles observed at 38 loci, no allele was exclusive to this species (Table 1). Intraspecific analysis was carried out on 34 loci in two samples. See Filippucci (1992) for allelic frequencies in individual populations. Six loci were polymorphic in the two Italian populations (Appendix 2).

A. uralensis

Sixty-six alleles were observed at 38 loci, four of them being species-specific (Table 1). Intraspecific analysis was carried out on 36 loci in 15 populations. Seventeen loci were monomorphic (*Sdh*, *Ldh-1*, *Ldh-2*, *Mdh-1*, *Mdh-2*, *Idh-1*, *Idh-2*, *Ipo-1*, *Ipo-2*, *Got-2*, *Hk-1*, *Hk-2*, *Ck*, *Adk*, *Lap*, *Aldo*, *Fum*). Additional loci were considered for interspecific comparisons: one of them (*Acph*) was monomorphic and one (*Adh*) was polymorphic. No allele was exclusive to Europe or Asia Minor.

A. cf. hyrcanicus

Of 40 alleles observed at 36 loci, two were exclusive to this taxon (Table 1). Intraspecific analysis was carried out on 36 loci in two population samples. Three loci were polymorphic (*Me-1*, *Ada*, *Est-3*).

A. mystacinus

In total, 71 alleles were observed at 38 loci in populations from the Middle East (*A. m. mystacinus*), 19 of them being exclusive (Table 1). In the single Balkan specimen from Galičica (*A. m. epimelas*), 36 alleles at 35 loci were observed, six of them being exclusive. Intraspecific analysis was carried out on 32 loci in seven populations. See Filippucci *et al*. (1989) for allelic frequencies in individual populations from Israel and Appendix 2 for mean allele frequencies in

the species. Fifteen loci were found to be monomorphic (*αGpdh*, *Sdh*, *Mdh-1*, *Mdh-2*, *Idh-2*, *G3pdh*, *Ipo-1*, *Ipo-2*, *Got-2*, *Hk-2*, *Adk*, *Lap*, *Aldo*, *Fum*, *Pgi*). Of the extra loci included in interspecific comparisons, two were monomorphic (*Adh*, *Pep-3*) and four were polymorphic (*Pgm-1*, *Acph*, *Pep-1*, *Pep-2*). Eight loci (*Ldh-1*, *Ldh-2*, *Me-2*, *Idh-1*, *G6pdh*, *Pep-1*, *Pep-2*, *Mpi*) were discriminant and four loci (*Me-1*, *6Pgdh*, *Est-1*, *Est-2*) were partially discriminant between the Balkan *A. m. epimelas* and Anatolian/Israeli populations of *A. m. mystacinus*.

A. agrarius

Forty-five alleles were observed at 37 loci in this species; 15 of them were species-specific (Table 1). Intraspecific analysis was carried out on 34 loci in two populations. Six loci were polymorphic (see Appendix 2). Filippucci (1992) describes allele frequencies in populations of this species.

A. peninsulae

Out of 35 alleles observed at 34 loci, four were exclusive (Table 1). The two specimens analysed showed polymorphism only at *Mpi*.

GENETIC SUMMARY

Mean values of genetic variation at the species level are given in Table 2. Values of genetic variation in *A. peninsulae* are only tentative, because of the small sample size. The overall mean number of alleles per locus ranged from 1.029 in *A. peninsulae* to 1.333 in *A. mystacinus*. The overall mean proportion of polymorphic loci ($P_{1\%}$) ranged from 0.029 in *A. peninsulae* to 0.306 in *A. m. mystacinus*. Mean observed heterozygosity was about 0.02 in *A. sylvaticus* and *A. peninsulae* up to about 0.04 in *A. flavicollis*, *A. uralensis*, *A. hermonensis*, *A. m. mystacinus*, and *A. agrarius*.

The highest mean values of genetic diversity were observed in *A. mystacinus* (H_e = 0.059, range 0.035–0.080) and in *A. flavicollis* (H_e = 0.045, range 0.000–0.094). Within populations of the latter species, the lowest diversity was found in those from central and southern Italy (H_e = 0.038, range 0.020–0.054) and from Asia Minor, Iran and Israel (H_e = 0.034, range 0.000–0.087). Alpine, northern-European and Balkan populations displayed higher mean values (H_e = 0.067, range 0.043–0.094).

In *A. hermonensis*, the highest mean values of genetic diversity were observed in populations from southern Iran (H_e = 0.069, range 0.028–0.089), whereas a much lower mean value (H_e = 0.03, range 0.000–0.059) was found in Turkey and Israel. In *A. uralensis*, observed heterozygosity averaged 0.04 in both European and Turkish populations. Except for *A. peninsulae* (H_e = 0.011), which was under-represented in the material under study (see Appendix 1), the lowest mean values of H_e were observed in *A. sylvaticus* (H_e = 0.020, range 0.000–0.069). In this species, the lowest diversity (H_e = 0.016, range 0.000–0.041) was found in populations from North Africa, the Iberian Peninsula and central Europe, whereas values nearly twice as high were apparent in Italian (both insular and peninsular populations, H_e = 0.029, range 0.011–0.052) and Balkan populations (H_e = 0.037, range 0.017–0.069).

Estimates of Wright's F-statistics are given in Table 3. Only samples of $N \geq 7$ individuals were considered for calculations. A slight deficiency of heterozygotes due to inbreeding within conspecific populations was found in *A. sylvaticus* and *A. flavicollis* (F_{IS} = 0.135 and 0.155, respectively) whereas virtually no inbreeding was apparent in *A. uralensis* (F_{IS} = 0.077) and *A. hermonensis* (F_{IS} = –0.008). The highest value of F_{IS} was observed in *A. m. mystacinus* (F_{IS} = 0.337). The mean value of F_{IT} ranged from 0.228 in *A. uralensis* to 0.432 in *A. flavicollis* and 0.435 in

Table 2. Values of some indices of genetic variation (*A*, mean number of alleles per locus; $P_{1\%}$, proportion of polymorphic loci under 1% criterion; H_o, mean observed heterozygosity; H_e, mean expected heterozygosity) and mean intraspecific values of Nei (1978) genetic distance within *Apodemus* species (*D*)

Species	No. loci	No. populations	No. individuals	H_o	H_e	$P_{1\%}$	*A*	*D*
A. sylvaticus	29	42	584	0.020	0.023	0.113	1.138	0.007
A. flavicollis	28	30	377	0.037	0.045	0.165	1.167	0.020
A. alpicola	33	2	18	0.031	0.033	0.136	1.166	0.015
A. hermonensis	36	20	98	0.037	0.040	0.107	1.100	0.019
A. uralensis	36	14	105	0.042	0.045	0.146	1.160	0.007
A. cf. *hyrcanicus*	36	2	8	0.028	0.027	0.069	1.070	0.000
A. m. mystacinus	32	6	146	0.038	0.059	0.306	1.333	0.011
A. peninsulae	34	1	2	0.015	0.011	0.029	1.029	–
A. agrarius	33	2	9	0.040	0.035	0.121	1.121	0.027

Table 3. Mean values of coefficients of Wright's F-statistics and estimates of the number of migrants exchanged between population samples under assumption of island model (*Nm* was approximated by the formula $Nm \approx (1/F_{ST} - 1)/4$; Wright, 1943). Only samples of N ≥ 7 were considered

Species	F_{IS}	F_{IT}	F_{ST}	*Nm*
A. sylvaticus	0.135	0.392	0.297	0.652
A. flavicollis	0.155	0.432	0.328	0.444
A. hermonensis	–0.008	0.252	0.258	0.394
A. uralensis	0.077	0.228	0.164	0.606
A. m. mystacinus	0.337	0.435	0.148	1.439

A. m. mystacinus. In the latter species, however, the high value of F_{IT} is largely due to intrapopulation inbreeding since the value of the fixation index is relatively low (F_{ST} = 0.148, the lowest value among the species studied) when compared with *A. flavicollis* (F_{ST} = 0.328, the highest value). Rather high values of F_{ST} observed in *A. flavicollis* (0.328), *A. sylvaticus* (0.297), and *A. hermonensis* (0.258) indicate that as much as about 26–33% of genetic variation in these species is due to differentiation among populations, thus indicating extensive interdemic genic differentiation within the species.

Intraspecific differentiation

Indices of genetic distance were calculated from allelic frequencies at 28 loci in *A. flavicollis*, 29 loci in *A. sylvaticus*, 32 loci in *A. mystacinus*, 33 loci in *A. alpicola* and *A. agrarius*, and 36 loci in *A. hermonensis*, *A. uralensis* and *A.* cf. *hyrcanicus*. Data for *A. alpicola*, *A. agrarius*, *A. hermonensis*, *A. uralensis*, and *A.* cf. *hyrcanicus* are the same as those published previously (Filippucci, 1992; Filippucci *et al.*, 1996; Macholán *et al.*, 2001a). Mean intraspecific values of genetic distance for each species are available upon request from one of the authors (MGF). Although, in general, Cavalli-Sforza & Edwards' (1967) chord distances performed better than Nei's genetic distances (both standard and unbiased) as revealed by higher cophenetic correlation coefficients (not shown here), Nei's (1978) distance will be presented in the following text since these are by far the most widely quoted, and our results thus can be more easily compared to those published elsewhere.

A. sylvaticus

The mean value of genetic distance among 42 populations of this species was 0.007, ranging from 0.000 to 0.049. The highest genetic distances were found between North African and European populations

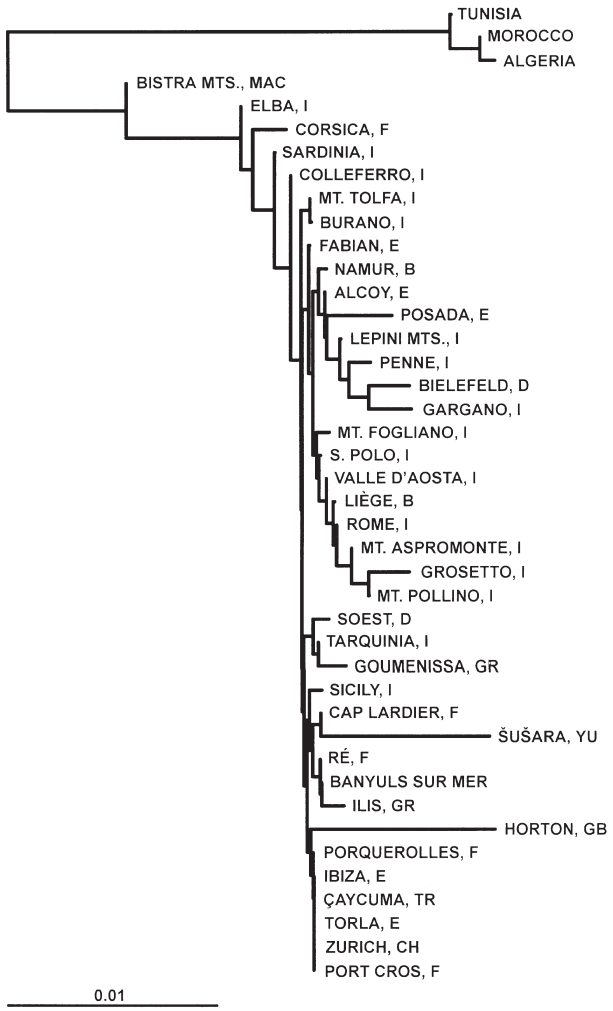

Figure 2. Neighbour-joining tree based on Nei (1978) unbiased distances between *A. sylvaticus* populations using 29 presumptive enzymatic loci. Note: the tree was constructed as unrooted but for convenience, it is shown as mid-rooted here.

(*D* = 0.036, 0.017–0.049), while the lowest values were found within the Italian samples (mean *D* = 0.002, 0.000–0.007). Genetic distances within North Africa and within Europe, were relatively low (0.000–0.015). Low distance values were also observed between insular Italian (Sicily, Corsica, Sardinia and Elba) and peninsular Italian populations (*D* = 0.003, ranging from 0.000 to 0.007). Genetic relationships among the populations sampled are shown in Fig. 2. There are two main groups in the tree, consisting of the African and European populations, respectively. These groups are genetically homogenous with the branching pattern within them being apparently random. The slightly distinct position of the Bistra population may be caused by the small sample size.

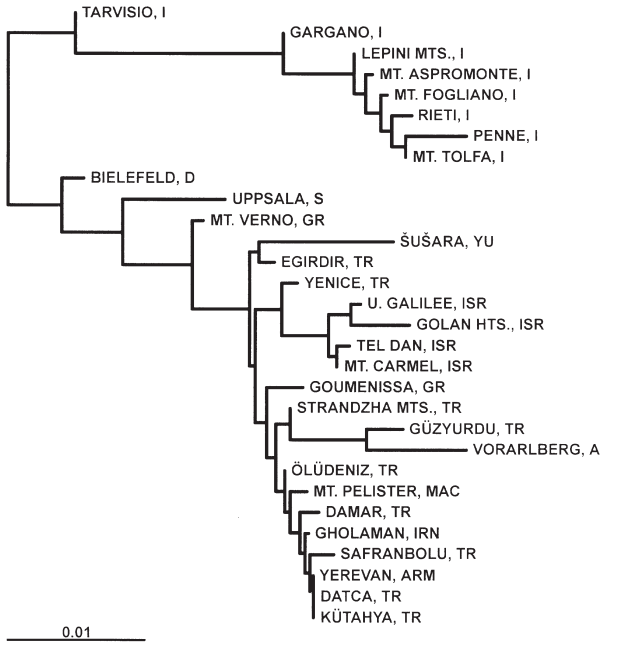

Figure 3. Unrooted neighbour-joining tree depicting interpopulation relationships in *A. flavicollis*, based on 28 loci. As in all following neigbor-joining trees, a matrix of Nei (1978) distances was used as input. The tree is shown as mid-rooted.

A. flavicollis

Among 30 populations of this species, the mean value of genetic distance was 0.020, ranging from 0.000 to 0.070. Low genetic differentiation was observed among populations from northern and central Europe, the Balkan Peninsula, Asia Minor, Iran and Israel (0.000–0.025). The highest value of genetic distance was observed between the Austrian population from Vorarlberg and the Israeli population from Tel Dan (D = 0.025). High homogeneity was also observed among Italian peninsular populations (D = 0.002). The northern Italian sample from Tarvisio displayed a mean value of genetic distance of 0.009 in comparison with peninsular populations. The highest values of genetic distance in *A. flavicollis* were found between peninsular populations from Italy and those from other European countries and the Middle East (D = 0.043). This is clearly illustrated in Fig. 3, where all the Italian populations appear to constitute a single clade, with the population from Tarvisio somewhat distinct from others as an apparent genetic transition between the Italian peninsula and the rest of Europe and the Middle East. The latter group (i.e. the Middle East and Europe except Italy) is rather homogenous except the 'northern' populations from Germany and Sweden.

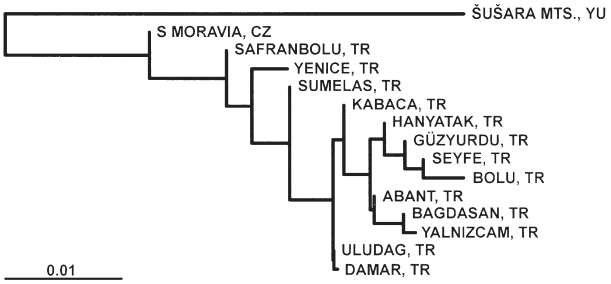

Figure 4. Neighbour-joining tree of *A. uralensis* populations, based on 36 enzymatic loci. The tree was mid-rooted for convenience.

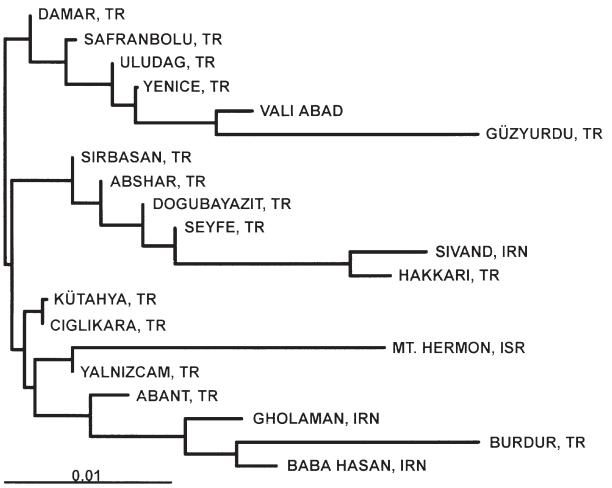

Figure 5. Mid-rooted neighbour-joining tree of *A. hermonensis* populations, based on 36 loci.

A. uralensis

The mean value of genetic distance among 14 populations of this species was low (D = 0.019, ranging from 0.000 to 0.098). The Mantel test revealed slight yet significant correlation between genetic distances and geographical distances (r = 0.433, $\Pr[Z_{rand} \geq Z_{obs}] = 0.068$). It is not clear if the distinct position of the Yugoslavian population (Fig. 4) is real or an artefact of small sample size (cf. Appendix 1). More importantly, the population of '*A. microps*' from southern Moravia (Czech Republic) appears a sister group of the Turkish clade.

A. hermonensis

The mean value of genetic distance among 20 populations of this species was 0.019, ranging from 0.000 to 0.059. Great heterogeneity in the rate of genetic change was revealed by the neighbour-joining tree (Fig. 5). As in *A. uralensis*, there is low coincidence between genetic and geographical distances in this

species (Mantel statistics: $r = 0.081$, $\Pr[Z_{rand} \geq Z_{obs}] = 0.165$).

A. alpicola
Only two populations of this species were studied (Collardente and Entreves, both in Italy) and the value of genetic distance between them was 0.015 (see Filippucci, 1992).

A. cf. hyrcanicus
The genetic distance between the two populations from northern Iran (Asalem and Now Kandeh) was 0.000 (Macholán *et al*., 2001a).

A. mystacinus
High values of genetic distance were observed between the two subspecies, European *A. m. epimelas* and *A. m. mystacinus* from Turkey and Israel (mean $D = 0.359$). Distances between Turkish and Israeli populations were much lower (mean $D = 0.028$, ranging from 0.026 to 0.031) (Fig. 6). There was strong correspondence between genetic and geographical distances in *A. m. mystacinus* (Mantel statistics: $r = 0.981$, $\Pr[Z_{rand} \geq Z_{obs}] = 0.106$).

A. agrarius
A relatively low value of genetic distance ($D = 0.027$) was observed among the two populations studied.

Interspecific differentiation and phylogenetic relationships

Several loci, showing fixation for alternative alleles, contributed to the discrimination of the species (Table 1). The lowest number of discriminant loci was found between *A. alpicola* and *A. flavicollis* (one, *Adh*, discriminant, and other three partially discriminant: *Me-1*, *Idh-1*, and *Np*) and *A. uralensis* (*Pep-1* discriminant and *αGpdh*, *Me-1*, *Np*, and *Est-2* partially discriminant). Between *A. flavicollis* and *A. uralensis*, two loci were discriminant (*Adh*, *Pep-1*) and another three (*αGpdh*, *Idh-1*, *Est-2*) partially discriminant. *Apodemus* cf. *hyrcanicus* and *A. uralensis* were discriminated by three loci (*Np*, *Est-2*, *Ada*), with *Me-1* partially discriminant. Between *A. flavicollis* and *A. hermonensis*, three loci were discriminant (*Ipo-2*, *Np*, and *Pep-2*) and one locus was partially discriminant (*Pep-1*). A larger number of discriminant loci were found between *A. sylvaticus* and other *Sylvaemus* species: five in comparison with *A. alpicola* (*αGpdh*, *Ldh-1*, *Me-2*, *Ipo-1*, *Pep-1*); six in comparison with *A. flavicollis* (*Adh*, *αGpdh*, *Ldh-1*, *Me-2*, *Ipo-1*, *Np*); seven with *A. hermonensis* (*Adh*, *αGpdh*, *Ldh-1*, *Me-2*, *Ipo-1*, *Ipo-2*, *Pep-2*), *A. uralensis* (*αGpdh*, *Ldh-1*, *Me-2*, *Ipo-1*, *Np*, *Est-2*, *Pep-1*), and *A.*cf. *hyrcanicus* (*αGpdh*, *Ldh-1*, *Me-2*, *Ipo-1*, *Np*, *Pep-1*, *Ada*).

The discrimination of *Karstomys* and *Sylvaemus* species is based on eight loci (between *A. m. mystacinus* and *A. sylvaticus*) up to 18 loci (between *A. m. epimelas* and both *A. alpicola* and *A. hermonensis*). Finally, the highest numbers of discriminant loci (19–26) were found comparing *A. agrarius* and *A. peninsulae* with *Sylvaemus* (20–26 loci) and *Karstomys* (19–24 loci) species.

Values of Nei's genetic distance (Nei, 1972, 1978), based on allele frequencies at 34 loci, are shown in Table 4. The lowest values of interspecific genetic distance were observed between *A. alpicola* and *A. flavi-*

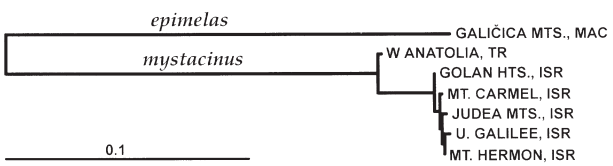

Figure 6. Mid-rooted neighbour-joining tree of populations of *A. mystacinus*, based on 32 enzymatic loci.

Table 4. Values of Nei (1972) standard genetic distance (below diagonal) and Nei (1978) unbiased genetic distance (above diagonal) among species of the genus *Apodemus*, based on 34 loci

Taxon	SYL	FLA	ALP	HER	URA	HYR	MYS	EPI	PEN	AGR
A. sylvaticus	–	0.222	0.166	0.237	0.238	0.244	0.539	0.675	1.116	1.101
A. flavicollis	0.222	–	0.065	0.126	0.121	0.174	0.433	0.646	1.055	1.178
A. alpicola	0.167	0.066	–	0.139	0.120	0.132	0.398	0.670	1.071	1.209
A. hermonensis	0.238	0.126	0.140	–	0.232	0.253	0.509	0.687	0.965	1.032
A. uralensis	0.238	0.122	0.121	0.232	–	0.108	0.378	0.664	0.913	1.341
A. cf. *hyrcanicus*	0.245	0.175	0.133	0.253	0.109	–	0.349	0.671	0.914	1.208
A. m. mystacinus	0.540	0.433	0.398	0.510	0.378	0.350	–	0.417	0.877	1.312
A. m. epimelas	0.675	0.646	0.671	0.687	0.664	0.671	0.417	–	0.954	1.186
A. peninsulae	1.118	1.057	1.073	0.967	0.915	0.917	0.879	0.956	–	0.619
A. agrarius	1.102	1.179	1.211	1.033	1.343	1.211	1.313	1.188	0.623	–

collis (Nei's unbiased distance $D = 0.065$) and between *A. uralensis* and *A.* cf. *hyrcanicus* ($D = 0.108$). The mean distance value between *Karstomys* and *Sylvaemus* was 0.552, ranging from 0.349 (*A. m. mystacinus* – *A. uralensis*) to 0.687 (*A. m. epimelas* – *A. hermonensis*). *Apodemus peninsulae* was genetically closest to *A. agrarius* ($D = 0.619$) while the mean genetic distance between these two species and *Sylvaemys–Karstomys* was 1.089, with *A. peninsulae* being closer to *Karstomys* ($D = 0.915$) than to *Sylvaemus* (D = 1.006). *Apodemus agrarius* always displayed distances greater than 1.1 in comparison with all other species.

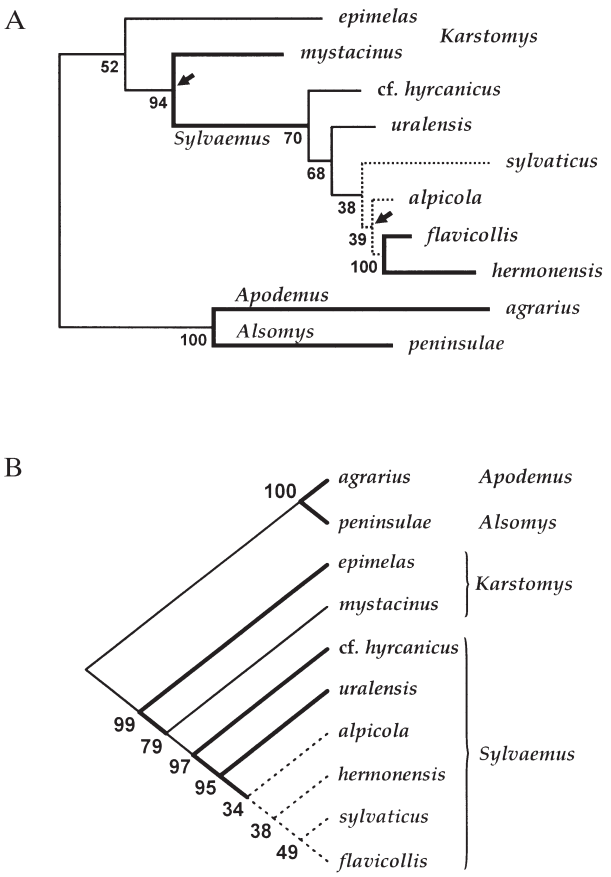

Figure 7. (A) Maximum-likelihood tree showing phylogenetic relationships between the *Apodemus* species studied. Bootstrap values at each node indicate percentage out of 1000 pseudoreplicated trees. Branches with strong support (bootstrap values ≥ 90) are in bold, branches with very weak support (bootstrap values ≤ 50) are shown as dotted lines. The tree is shown as mid-rooted. Arrows indicate branches that are not significantly different from zero. (B) Wagner parsimony tree based on 'mutation coding scheme' of Murphy (1993) and Murphy & Doyle (1998). Numbers indicate bootstrap support after 1000 pseudoreplications.

There was general disagreement in the branching pattern of phylogenetic trees revealed by the four different methods employed. This is illustrated by low bootstrap support for most of the nodes (Fig. 7). Nevertheless, there were a few branching patterns common to all or the majority of trees. First, *A. agrarius* and *A. peninsulae* formed a well-defined clade, distinct from other species. Second, *A. m. epimelas* and *A. m. mystacinus* appeared highly differentiated from each other, and indeed these taxa did not even form a monophyletic group in half of the trees. Third, the two *Karstomys* taxa were sister species to the *Sylvaemus* group *sensu stricto*. And finally, *A.* cf. *hyrcanicus* and *A. uralensis* appeared to be sister species to the *A. sylvaticus–flavicollis–alpicola–hermonensis*. It is also of some interest that the rates of allozyme evolution appeared quite uneven among lineages, and *A. agrarius*, *A. sylvaticus* and *A. hermonensis* showed the highest rates in all three phylogenetic methods taking this parameter into account (i.e. maximum-likelihood, Fitch-Margoliash, and FREQPARS parsimony; see the ML tree in Fig. 7). A majority-rule consensus tree constructed from the four trees is shown in Fig. 8. Numbers in parentheses indicate how many times each clade appeared in individual trees. Again, well-supported clades are formed by *A. agrarius–A. peninsulae* and *Sylvaemus* with *A. mystacinus/epimelas* as

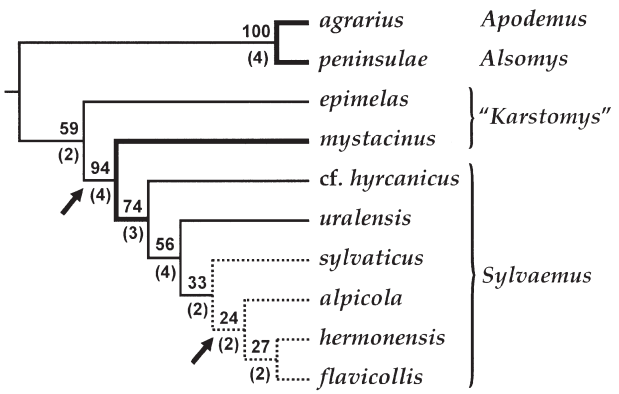

Figure 8. Majority-rule consensus tree based on phylogenies inferred by four different methods (maximum-likelihood; Fitch–Margoliash procedure on Nei (1972) standard distances; character-based Wagner parsimony; and Swofford & Berlocher's method). Numbers in parenthesses indicate how many times respective nodes appeared in the four trees; bootstrap values are shown above them, indicating percentage out of 3000 trees (three data sets, each consisting of 1000 pseudoreplicated trees, were pooled, excluding Swofford & Berlocher's parsimony method). Branches with strong support (bootstrap values ≥ 90) are in bold, branches with very weak support (bootstrap values ≤ 50) are shown as dotted lines. Note: since not all methods were used for bootstrapping, the numbers can be biased.

a sister group to *A.* cf. *hyrcanicus*, *uralensis*, *sylvaticus*, *alpicola*, *hermonensis*, *flavicollis*.

DISCUSSION

The values of genetic variation observed in *Apodemus* are within the range generally reported for this genus (e.g. Gemmeke, 1980; Mezhzherin, 1990; Britton-Davidian *et al*., 1991), and for other rodents in general (Nevo *et al.*, 1990). According to Selander (1976), marginal populations may display lower values of genetic variation, as a consequence both of founder effect and genetic drift. This may be the case for populations of *A. flavicollis* from Italy, representing the southern border of the European distribution, and from Iran and Israel, representing the south-eastern border of the species distribution. Moreover, in peninsular Italy the populations of this species are stenotopic, localized and sometimes isolated, mostly inhabiting coniferous and broadleaf deciduous forests.

We attempted to compensate for small sample size by analysing a large number of loci. Values of heterozygosity (and genetic distance) are therefore reliable with a reasonable margin of precision according to Nei (1978), Gorman & Renzi (1979), and Sage *et al*. (1986) (but see Archie *et al.*, 1989 for a different opinion).

According to Wright's (1978) suggestions (see also Hartl & Clark, 1997), moderate to very high genetic differentiation among populations within *Apodemus* species was indicated by F_{ST} values (range 0.148–0.328). Estimates of the number of migrants exchanged between populations per generation (Nm), can be approximated by the formula $F_{ST} \approx 1/(4Nm + 1)$ (Wright, 1943), and these ranged from an average of 0.394 in *A. hermonensis*, 0.444 in *A. flavicollis*, 0.606 in *A. uralensis*, 0.652 in *A. sylvaticus*, to 1.439 in *A. mystacinus*. These values suggest lower levels of gene flow in some species, with about one migrant every second generation exchanged in *A. sylvaticus* and *A. flavicollis*, whereas more than one migrant every generation is exchanged in *A. mystacinus* (*cf.* Table 3, right column). It should be noted, however, that the above equation is based on the island model of migration, and it is not clear to what extent the results based on this simple model can be considered reliable.

The mean values of genetic distance among populations are within the range generally observed in other rodent species (Zimmermann *et al.*, 1978; Graf, 1982). Low values observed among European populations of *A. sylvaticus* (D = 0.003; range 0.000–0.015) may be due to the eurytopic niche of this species, favouring gene flow between contiguous populations. Insular populations (Sicily, Corsica, Sardinia and Elba) appeared genetically close to those from the peninsula (Fig. 2). However, mtDNA studies suggested that *A. sylvaticus* is divided into three well-differentiated groups, the first comprising all western European populations from Spain to Scandinavia, the second consisting of those from Italian and Tyrrhenian islands, and the third group consisting of animals from Sicily (Michaux *et al*., 1998a,b; Libois *et al*., 2001). This discordance may be a result of different evolutionary rates between mitochondrial and nuclear markers. Under the assumption of neutrality, the expected time for lineage sorting in two idealized and isolated populations is four times longer for nuclear markers than for mitochondrial ones (Avise, 2000). Moreover, in small mammals, it is usually males who are characterized by higher dispersal rates in comparison to more philopatric females. Thus certain mtDNA genes may reflect, with higher fidelity, deeper evolutionary relationships, while biparentally inherited allozymes may reflect recent gene flow among wood mouse populations. Finally, reduced sensitivity of protein electrophoresis at the intraspecific level cannot be ruled out, although in other cases it has proven to be suitable in distinguishing phylogroups within species (e.g. African vs. other populations of *A. sylvaticus*; Italian vs. other populations of *A. flavicollis*; *cf.* Figs 1, 2).

The population of *A. sylvaticus* endemic to Elba, being morphologically well-differentiated and characterized by increased body size (Kahmann & Niethammer, 1971; Filippucci *et al*., 1984), appeared to be genetically close to mainland populations. The morphological differentiation may be due to selective pressures rather than to founder effect and/or genetic drift (Michaux *et al*., 1996a). The low genetic distance between Sardinia and Corsica on the one hand, and mainland populations on the other is in agreement with the recent origin of insular populations of *A. sylvaticus*. According to Vigne (1990), this species, as well as other small mammals, appeared in Sardinia and Corsica during the late Neolithic period. Because of the preclusion of land passage between these two islands and the mainland during the Holocene, the presence of these species is probably due to the intensity of shipping since the Neolithic. Analysis of mtDNA restriction patterns (Michaux *et al*., 1996a) confirmed the anthropogenic origin of wood mice from Sardinia, Corsica and Elba islands. Whereas Sardinia was probably invaded directly from Italy, our results suggest that the Corsican populations originated from Etruria through Elba, as indicated by the presence of allele *Got-2*^{92} on the latter two islands.

Although genic and morphologic evolution are generally thought to be independent in mammals (Schnell & Selander, 1981), the comparison of mean values of genetic distance displayed by populations of *A. sylvaticus* with those observed among subspecies in other rodents (D = 0.052, Zimmermann *et al*., 1978; D = 0.064, Graf, 1982) should lead to a critical revision

of the subspecific division of this species. However, as stated above, allozyme analysis may not be sensitive enough to clarify the taxonomy of *A. sylvaticus*. According to RFLP studies by Michaux *et al*. (1996a, 1998a), populations from Spain to north-western Europe belong to the same subspecies, *A. s. sylvaticus*. The origin of these populations on the Iberian Peninsula was also hypothesized by Gemmeke *et al.* (1987). According to these authors, *A. sylvaticus* disappeared from south-central Europe during the Upper Pleistocene glacial periods and recolonized this area and northern Europe during the Holocene. Populations on Tyrrhenian islands and peninsular Italy (subspecies *A. s. milleri*) have a common origin and differ from the north-western subspecies, suggesting that the Alps may act as a biogeographical barrier (Michaux *et al.*, 1996b). Finally, the populations from Sicily are distinctive from the rest of Europe and can be considered a separate subspecies, *A. s. dichrurus* (Michaux *et al*., 1998b).

An open question remains about the affinity of Balkan populations. According to Dulić & Tvrtković (1974) there is considerable confusion concerning the status of *A. sylvaticus* in this area. High morphological variation was observed in this species and several taxa were described for this region. Therefore, a detailed analysis of Balkan populations is needed.

Between North African populations, low values of genetic distance were observed ($0.000 < D < 0.001$), supporting the opinion of Kowalski & Rzebik-Kowalska (1991) that there are no differences among the populations, which are attributed to the subspecies *A. s. hayi*. The distance between this taxon and European populations ($D = 0.036$; 0.017–0.049) corresponds to distances generally observed among subspecies in other rodents. This genetic homogeneity has also been reflected in a mtDNA restriction pattern study (Libois *et al*., 2001) and confirms the presence of only a single subspecies in North Africa. According to Gemmeke *et al*. (1987), the Tunisian wood mice probably originated from south-western Europe, and this hypothesis was supported by Filippucci (1992) and Michaux *et al*. (1996b, 1998b).

Populations of *A. flavicollis*, which as a species appears more stenotopic than *A. sylvaticus*, are slightly more differentiated. Reduced gene flow between populations (especially within peninsular Italy, where the populations are mostly limited to the Apennine region) results in an increased degree of morphological and biochemical differentiation (Filippucci *et al*., 1984; Nascetti & Filippucci, 1984). The highest values of genetic distance were observed between Italian populations from the Apennine Peninsula ascribed to the subspecies *A. f. geminae*, and those from the rest of Europe and the Middle East ($D = 0.043$, 0.006–0.066). Two loci (*6Pgdh*, *Est-3*) contributed to the differentiation of the peninsular populations, displaying substantial differences in allelic frequencies. The genetic affinity between Middle Eastern and Balkan populations is in agreement with Tchernov's opinion that the genus *Apodemus* has probably invaded the Middle East from southern Europe relatively recently (Tchernov, 1979).

Relatively high differentiation ($D = 0.417$) was found between *A. m. epimelas* from the Balkans and from *A. m. mystacinus* of the Middle East. Although our analysis is based on a single specimen of *epimelas* from Galičica, the high value of genetic distance observed and the large number of new alleles at several loci suggest the two taxa could represent two distinct species. This result corroborates the hypothesis of an ancient separation between *epimelas* and *mystacinus*, dating to the Pleistocene (Storch, 1977).

In interspecific comparisons, the lowest genetic distances were found between *A. flavicollis*, *A. alpicola*, and *A. hermonensis* ($0.060 < D < 0.139$), and between *A. uralensis* and *A.* cf. *hyrcanicus* from northern Iran ($D = 0.108$; the mean value between these two groups was 0.172). The allozyme data suggest a recent radiation of species within *Sylvaemus* (in the narrow sense, i.e. excluding *A. mystacinus*).

As discussed in Macholán *et al*. (2001a), the taxonomic position of south-eastern taxa is problematic and should be further investigated. *A. hermonensis* was described from Mt. Hermon in Israel, yet it appears to be a common species in both Asia Minor and Iran (Filippucci *et al*., 1996; Macholán *et al*., 2001a). According to the analysis of morphological characters, *A. hermonensis* is probably a junior synonym of *A. fulvipectus* (Filippucci *et al*., 1996). Populations of this species from the Middle East morphologically and ecologically correspond to the taxon *chorassanicus*, recently included in *A. fulvipectus* by Musser & Carleton (1993), and this is further corroborated by comparison of present data with those of Mezhzherin, 1990) and Lavrenchenko & Likhnova (1995). Diagnostic biochemical characters for *A. fulvipectus* are the presence of discriminant alleles at *Sdh*, *Sod-2* (Mezhzherin, 1990), *Hb* and *Np* (Lavrenchenko & Likhnova, 1995). According to our data, *Np* and *Ipo-2* (= *Sod-2*) discriminate *A. hermonensis* from *A. flavicollis*, *A. uralensis*, *A.* cf. *hyrcanicus*, and *A. alpicola*.

Populations from Nepal and Iran, previously attributed to *A. sylvaticus* using morphological characters, displayed a higher affinity with *A. flavicollis* than with *A. sylvaticus* when studied electrophoretically, although they were differentiated from both of them (Darviche *et al*., 1979; Gemmeke & Niethammer, 1982). Recently, Macholán *et al*. (2001a), documented the presence of three species in Iran (*A. hermonensis*, *A. flavicollis*, *A.* cf. *hyrcanicus*), of which *A. hermo-*

nensis was the most widespread. Mezhzherin (1997b) and Zagorodnyuk *et al*. (1997) synonymized the taxa *falzfeini*, *chorassanicus*, *fulvipectus*, and *hermonensis* with Iranian *arianus*, the latter taxon being the oldest known synonym. Recently, Kryštufek & Vohralík (2001) and Kryštufek (2002), on morphological criteria, have asserted *Apodemus iconicus* Heptner, 1948 to be a valid older synonym of *A. hermonensis*. Nonetheless, the relationship of the Middle Eastern *A. flavicollis* to the Caucasian *A. ponticus*, the affinity of *Apodemus* sp. (provisionally named *A*. cf. *hyrcanicus* in Macholán *et al*. (2001a) and in this paper) to *A. hyrcanicus* described from Talysh (Mezhzherin *et al*., 1992; Vorontsov *et al*., 1992), and the systematic status of *A. arianus* and *A. wardi* remain unclear.

The mean value of genetic distance between *A. sylvaticus* and other *Sylvaemus* species was 0.22, indicating that the separation from a common ancestor occurred approximately one million years ago (according to Nei's (1975) formula, $T = 5 \times 10^6 D$). This estimate is in agreement with fossil records (Michaux & Pasquier, 1974), as well as with the results of rDNA analysis by Suzuki *et al*. (1990), although it is about four times higher than the allozyme-based estimate of Gebczyński *et al.* (1986). Conversely, this is two to four times lower than estimates of Serizawa *et al*. (2000) based on nucleotide sequence data.

Within *Sylvaemus* (excluding *mystacinus* and *epimelas*), the phylogenetic relationships are unresolvable by protein electrophoresis, partly owing to rapid adaptive radiation of the group and partly because of limitations of the method. The branching patterns presented in this paper differ from those published previously (e.g. Mezhzherin, 1990; Filippucci, 1992; Filippucci *et al*., 1996; *cf.* Fig. 8) mainly in the placement of *A. sylvaticus*, which has usually been outside other species. However, previous allozyme studies almost exclusively used the UPGMA for depicting genetic relationships among species of *Apodemus*, yet it has been shown that the method is inappropriate when there are deviations from ultrametricity (i.e. when the assumption of a molecular clock is violated; see Swofford *et al*., 1996 for details). It is also known that taxa with presumed higher rates of genic change tend to appear most distant in UPGMA phenograms, as do, for example, *A. sylvaticus*, *A. hermonensis* and *A. agrarius* in this paper (UPGMA trees not shown here).

The genetic differentiation between *A. mystacinus/epimelas* and *Sylvaemus* corresponds to that generally observed between morphologically well-differentiated species of small mammals. Although the former pair of species have appeared genetically diversified from the latter group (e.g. Britton-Davidian *et al*., 1991; Filippucci, 1992; Mezhzherin, 1997a; this paper), the validity of *Karstomys* remains unclear.

Apodemus peninsulae appeared closer to *A. agrarius* than to *A. mystacinus/epimelas* and other *Sylvaemus* species. This corresponds to Mezhzherin & Zykov (1991), Bellinvia *et al*. (1999), and Serizawa *et al*. (2000) (but see Suzuki *et al*., 1990). Musser *et al*.'s (1996) decision of including *A. agrarius* and *A. peninsulae* in the same *Apodemus*-group thus seems appropriate. High genetic distances between *A. agrarius*/*A. peninsulae* and other species (D = 1.10–1.32) are similar to those generally observed among different genera of rodents. For instance, Graf (1982) found an average value of D = 0.75 within Arvicolidae, Honeycutt & Williams (1982) found D = 1.01 in Geomyinae, and Filippucci & Kotsakis (1995) reported D = 1.69 in Myoxidae. High values of genetic distance between *A. agrarius* and other European species of *Apodemus* have been reported in the majority of papers investigating allozyme variation in these species. Moreover, the subgenus *Apodemus* did not appear to be more closely related to *Sylvaemus* than to *Mus* and/or *Rattus* (Bonhomme *et al*., 1985; Mezhzherin, 1997a). However, here we are faced with the same problem as discussed in the case of *A. sylvaticus* (see above) of allozyme evolution within lineages, as was suggested by Hartl *et al*. (1992). According to these authors paraphyly of the genus *Apodemus*, although supported by the UPGMA clustering method based on the results of allozyme studies, is not substantiated when the parsimony method is applied to the data. Unfortunately as no outgroup was used in this study we cannot resolve this problem even though appropriate methods of phylogenetic inference, including two parsimony approaches, were used. Including more *Apodemus* taxa from south-eastern Asia and the Far East as well would be desirable.

CONCLUSIONS

From the results obtained from the electrophoretic analysis of 1347 specimens of ten wood mice taxa from Europe, the Middle East and North Africa, we conclude that:

1 Of 38 loci scored, a single locus, *Lap*, was found to be monomorphic and fixed for the same allele, while rare (private) alleles appeared at three other loci, *Mdh-2*, *Ck* and *Adk*, at frequencies less than 1%. The taxa investigated had from 11 (between *A. agrarius* and *A. uralensis*, *A*. cf. *hyrcanicus*, *A. alpicola*) to 46 (between *A. sylvaticus* and *A. flavicollis*) common alleles.

2 The mean number of alleles (A) per locus ranged from 1.029 to 1.333; high variation was found in the mean proportion of polymorphic loci ($P_{1\%}$ =

0.029–0.306). Mean observed heterozygosity (H_o) ranged from about 2% (*A. sylvaticus*, *A. peninsulae*) to about 4% (*A. flavicollis*, *A. uralensis*, *A. hermonensis*, *A. m. mystacinus*, *A. agrarius*). The highest values of genetic diversity were found in *A. mystacinus* (H_e = 0.059) and *A. flavicollis* (H_e = 0.045).

3 Estimates of Wright's F-statistics, measured in samples of $N \geq 7$ only, ranged from F_{IS} = –0.008 (*A. hermonensis*) to F_{IS} = 0.155 (*A. flavicollis*), F_{IT} = 0.228 (*A. uralensis*) to F_{IT} = 0.435 (*A. m. mystacinus*), and F_{ST} = 0.148 (*A. m. mystacinus*) to F_{ST} = 0.328 (*A. flavicollis*).

4 Genetic distances were generally low between populations within individual species, the only exception being the distance between the two subspecies of *A. mystacinus*, European *epimelas* and *mystacinus* from the Middle East (Nei's D = 0.359), leading us to suggest the specific status for these taxa. Regardless of a rather random genetic pattern within species, several apparent discontinuities were revealed by neighbour-joining analyses in some species. First, populations of *A. sylvaticus* from North Africa constitute a separate lineage, clearly distinct from Europe. Second, Italian populations of *A. flavicollis* are distinguished from those of the rest of Europe and the Middle East, with the population from Tarvisio being intermediate between the two clades. Conversely, even though the southern Moravian population of '*A. microps*' appeared somewhat differentiated from those of *A. uralensis* from Asia Minor, their inclusion within the same species seems well substantiated.

5 *Apodemus agrarius* and *A. peninsulae* are sister taxa and should be included in the same group of species, as already suggested by Musser *et al*. (1996).

6 *Apodemus mystacinus* and *A. epimelas* appear sister taxa to other species of the *Sylvaemus* group, in agreement with conslusions of Musser *et al*. (1996) and Serizawa *et al*. (2000), although validity of the subgeneric name *Karstomys* for the former two taxa remains unclear.

7 Within the *Sylvaemus* group in the narrow sense (i.e. excluding *A. mystacinus* and *A. epimelas*), the phylogenetic relationships between species are unclear as the four different phylogenetic methods yielded incongruent results. However, *A.* cf. *hyrcanicus* and *A. uralensis* appeared outside the group *A. sylvaticus–A. flavicollis–A. alpicola–A. hermonensis* in the majority of trees.

To resolve the last point, more data (and possibly nucleotide sequences) are needed. However, even additional molecular data and genealogies may not resolve the *Sylvaemus* phylogeny since a mosaic neutral character evolution may result from rapid speciation events and radiation of the wood mice species. Further investigation is necessary for *A. hyrcanicus*, *A. fulvipectus*, *A. arianus*, *A. wardi* and *A. ponticus* from type localities, as well as other species from central Asia (such as *A. rusiges*, endemic to north India: Musser & Carleton, 1993), and from south-eastern European taxa *vohlynensis*, *mosquensis*, and *ciscaucasicus* (Orlov *et al*., 1996). A comparison between European and Asiatic species (especially with *A. argenteus*, presently included in a separate group of species) with an appropriate outgroup will be necessary for correct inference of the evolutionary relationships within the genus and to test the alternative hypotheses of whether *Apodemus* is monophyletic or polyphyletic.

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APPENDIX 1

LIST OF COLLECTING SITES, POPULATION ACRONYMS, AND NUMBER OF SPECIMENS ANALYSED.

See also Macholán *et al*. (2001a) for more details on Turkish localities.

Species/Localities		Code	*N*
A. sylvaticus			
Morocco:	Forêt de la Mamora (2), Bab Berred (5), Ketama (7), Ouezzane (2), Mt. Zerhoun (1), Cap Spartel (1)	SMOR	18
Algeria:	Akfadou (5), Costantina (1), El Milia (1), Mt. Edough (2)	SALG	9
Tunisia:	Aindram (6), Zaguan (3)	STUN	9
Spain:	Ibiza	SIBI	1
	Posada	SPOS	12
	Alcoy	SALC	15
	Fabian	SFAB	4
	Torla	STOR	2
France:	Ré	SRE	4
	Cap Lardier	SCPL	7
	Port Cros	SPCR	7
	Porquerolles	SPOR	12
	Banyuls sur Mer	SBAN	12
	Corsica	SCOR	23
Belgium:	Liège	SLIE	9
	Namur	SNAM	3
Switzerland:	Zurich	SZUR	2
Germany:	Soest	SSOE	4
	Bielefeld	SBIE	25
England:	Horton	SHOR	2
Italy:	Sicily: Palermo (2), Madonie Mts. (26), Mt. Etna (8)	SSIC	36
	Mt. Aspromonte, Calabria	SASP	21
	Mt. Pollino, Basilicata	SPOL	3
	Gargano, Apulia	SGAR	4
	Penne, Abruzzo	SPEN	44
	Lepini Mts., Latium	SLEP	46
	Colleferro, Latium	SCLF	31
	Rome, Latium	SROM	13
	S. Polo dei Cavalieri, Latium	SSPC	6
	Mt. Fogliano, Latium	SMFO	6
	Tolfa Mts., Latium	STOL	28
	Tarquinia, Latium	STRQ	21
	Burano, Tuscany	SBUR	91
	Grosseto, Tuscany	SGRO	4

Species/Localities		Code	*N*
	Elba Island, Tuscany	SELB	13
	P. Tricoli, Sardinia	SSAR	8
	St. Pierre, Valle d'Aosta	SVAO	3
Yugoslavia:	Šušara, Vojvodina	SSUS	16
Macedonia:	Bistra Mts.	SBIS	2
Greece:	Goumenissa, Macedonia	SGOU	2
	Ilis, Peloponnesus	SILI	3
Turkey:	Çaycuma, Zonguldak	SCAY	1
A. flavicollis			
Italy:	Mt. Aspromonte, Calabria	FASP	51
	Gargano, Apulia	FGAR	36
	Penne, Abruzzo	FPEN	9
	Lepini Mts., Latium	FLEP	70
	Tolfa Mts., Latium	FTOL	24
	Mt. Fogliano, Latium	FMFO	9
	Rieti, Latium	FRIE	6
	Tarvisio, Friuli	FTAR	25
Austria:	Thüringerberg, Vorarlberg	FVOR	12
Germany:	Bielefeld, Wuppertal, North Rhine	FBIE	3
Sweden:	Uppsala	FUPS	5
Yugoslavia:	Šušaara, Vojvodina	FSUS	5
Macedonia:	Mt. Pelister	FPEL	7
Greece:	Goumenissa, Macedonia	FGOU	3
	Mt. Verno, Epirus	FMVE	4
Turkey:	Strandzha Mts., Kirklareli, Thrace	FIST	16
	Safranbolu, Zonguldak	FSAF	2
	Yenice, Zonguldak	FYEN	8
	Göksuyu river, Kütahya	FKUT	1
	Eğirdir, Isparta	FEGR	7
	Datca, Marmaris	FDAT	5
	Ölüdeniz, Fethiye, Muðla	FOLD	4
	Güzyurdu, Gümüshane	FGUZ	2
	Damar, Artvin	FDAM	2
Armenia:	Echmiadzin, Yerevan	FYER	2
Iran:	Gholaman, Khorram Abad, Lorestan	FGHO	7
Israel:	Golan Heights	FGOL	4
	Tel. Dan	FTLD	15
	Upper Galilee	FUGA	14
	Mt. Carmel	FMCA	19
A. uralensis			
Czech Rep.	Vrbovec, Znojmo, S. Moravia	UMOR	12
Yugoslavia:	Šušara	USUS	2
Turkey:	Uludağ Mts., Bursa	UUDG	16
	Bolu	UBOL	6
	Lake Abant, Bolu	UABA	4
	Hanyatak, Sakarya	UHAN	5
	Yenice, Zonguldak	UYEN	6
	Safranbolu, Zonguldak	USAF	13
	Güzyurdu, Gümüshane	UGUZ	2
	Yalnizçam Pass, Artvin	UYAL	4
	Bağdaşan, Kars	UBGD	3
	Seyfe, Amasya	USFE	7
	Damar, Artvin	UDAM	16
	Kabaca, Artvin	UKBC	6
	Sumelas, Trabzon	USUM	5

A. cf. *hyrcanicus*			
Iran:	Asalem, Gilan	ASLM	6
	Now Kandeh, Mazandaran	NOWK	2
A. hermonensis			
Israel:	Mt. Hermon	HHER	12
Turkey:	Uludağ, Bursa	HUDG	3
	Lake Abant, Bolu	HABA	1
	Yenice, Zonguldak	HYEN	1
	Safranbolu, Zonguldak	HSAF	5
	Güksuyu river, Kütahya	HKUT	10
	Ciglikara, Antalya	HCIG	20
	Güzyurdu, Gümüshane	HGUZ	2
	Yalnizçam Pass, Artvin	HYAL	4
	Seyfe, Amasya	HSFE	1
	Damar, Artvin	HDAM	3
	Sirbasan, Kars	HSBS	9
	Hâkkari	HHAK	1
	Doğubayazit, Ağri	HDOG	1
	Burdur, Isparta	HBUR	1
Iran:	Gholaman, Khorram Abad, Lorestan	HGHO	8
	Baba Hasan, Boyerahmad-va-Kuhgiluyeh	HBHS	10
	Abshar, Fars	HABS	2
	Sivand, Fars	HSIV	1
	Vali Abad, Mazandaran	HVAL	1
A. m. mystacinus			
Turkey:	Safranbolu, Zonguldak	MSAF	12
	Ciglikara, Antalya	MCIG	1
	Ölüdeniz, Fethiye, Muğla	MOLD	4
Israel:	Mt. Hermon	MHER	19
	Golan Heights	MGOL	33
	Upper Galilee	MUGA	40
	Mt. Carmel	MMCA	27
	Hirbet Se'Adim, Judea Mts.	MJUD	12
A. m. epimelas			
Macedonia:	Galičica Mts.	MGAL	1
A. alpicola			
Italy:	Collardente, Liguria	ALIG	11
	Entreves, Valle d'Aosta	AVAO	7
A. agrarius			
Italy:	S. Eulalia, Treviso, Venetia	AGIT	5
Yugoslavia:	Šušara, Vojvodina	AGYU	4
A. peninsulae			
Russia:	Lake Baikal, Siberia	PEN	2

APPENDIX 2

ALLELIC FREQUENCIES IN *APODEMUS* SPECIES

Locus	SYL	FLA	ALP	HER	URA	HYR	MYS	EPI	PEN	AGR
Adh										
100	0.90	–	1.00	–	0.96	–	–			
98	0.07	–	–	–	–	–	–			
94	0.03	–	–	0.03	0.04	–	–			
92	–	1.00	–	0.93	–	–	–			
90	–	–	–	–	–	–	1.00			
85	–	–	–	–	–	1.00	–			
83	–	–	–	0.04	–	–	–			
αGpdh										
110	–	–	–	–	–	–	1.00	1.00	–	–
108	0.04	–	–	–	–	–	–	–	–	1.00
106	–	–	–	–	0.93	1.00	–	–	1.00	–
104	0.07	–	–	–	–	–	–	–	–	–
102	–	1.00	1.00	0.99	0.04	–	–	–	–	–
100	0.89	–	–	–	–	–	–	–	–	–
97	–	–	–	–	0.03	–	–	–	–	–
93	–	–	–	0.01	–	–	–	–	–	–
Sdh										
105	–	–	–	–	–	–	1.00	1.00	–	–
100	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	–
95	–	–	–	–	–	–	–	–	1.00	1.00
Ldh-1										
108	–	–	–	–	–	–	0.02	–	–	–
104	–	1.00	1.00	1.00	1.00	1.00	0.98	–	–	–
100	0.98	–	–	–	–	–	–	–	–	–
90	0.02	–	–	–	–	–	–	–	1.00	1.00
85	–	–	–	–	–	–	–	1.00	–	–
Ldh-2										
115	–	–	–	–	–	–	–	1.00	–	–
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–
97	–	–	–	–	–	–	–	–	1.00	1.00
Mdh-1										
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–
93	–	–	–	–	–	–	–	–	1.00	1.00
Me-1										
120	–	–	–	–	–	–	0.07	–	–	–
115	–	–	–	–	–	–	0.92	–	–	–
112	–	–	–	–	–	–	0.01	1.00	–	0.11
110	–	0.01	0.19	–	–	–	–	–	–	–
108	–	–	–	–	–	–	–	–	–	0.89
105	0.17	0.23	0.45	0.15	0.26	–	–	–	–	–
100	0.83	0.72	0.33	0.85	0.74	0.19	–	–	1.00	–
93	–	0.04	0.03	–	–	0.81	–	–	–	–
Me-2										
103	–	–	–	–	–	–	–	1.00	–	–
100	1.00	–	–	–	–	–	–	–	–	–
98	–	–	–	0.04	0.01	–	–	–	–	–
97	–	–	–	–	–	–	0.08	–	–	–
94	–	0.93	0.92	0.96	0.98	1.00	0.92	–	–	–
88	–	0.07	0.08	–	0.01	–	–	–	–	–
84	–	–	–	–	–	–	–	–	–	1.00
80	–	–	–	–	–	–	–	–	1.00	–

Idh-1										
115	–	–	–	–	–	–	–	–	1.00	1.00
108	0.02	0.98	0.03	1.00	–	–	–	–	–	–
104	–	–	–	–	–	–	–	1.00	–	–
100	0.98	0.01	0.97	–	1.00	1.00	1.00	–	–	–
90	–	0.01	–	–	–	–	–	–	–	–
Idh-2										
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94
96	–	–	–	–	–	–	–	–	–	0.06
6Pgdh										
115	–	–	–	–	–	–	0.02	1.00	–	0.33
112	–	–	–	0.01	–	–	–	–	–	–
110	–	–	–	–	–	–	0.92	–	–	–
108	–	0.01	–	0.03	–	–	–	–	–	–
100	0.99	0.68	0.97	0.96	0.53	1.00	–	–	1.00	0.67
98	–	–	–	–	–	–	0.06	–	–	–
92	0.01	0.31	0.03	–	0.47	–	–	–	–	–
G6pd										
104	–	–	–	–	–	–	–	–	1.00	–
100	1.00	1.00	1.00	1.00	1.00	1.00	–	1.00	–	1.00
96	–	–	–	–	–	–	0.99	–	–	–
92	–	–	–	–	–	–	0.01	–	–	–
G3pd										
105	–	–	–	–	0.01	–	–	–	–	–
100	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00
Ipo-1										
115	–	–	–	–	–	–	–	–	–	1.00
110	0.01	–	–	–	–	–	–	–	–	–
100	0.99	–	–	–	–	–	–	–	–	–
82	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–
Ipo-2										
105	–	–	–	–	–	–	–	–	–	1.00
100	1.00	1.00	1.00	–	1.00	1.00	1.00	1.00	–	–
97	–	–	–	1.00	–	–	–	–	1.00	–
Np										
105	–	–	–	–	–	–	1.00	1.00	–	–
104	0.01	–	–	–	–	–	–	–	–	–
103	–	–	–	–	–	–	–	–	1.00	–
100	0.99	–	0.86	1.00	–	–	–	–	–	–
97	–	–	–	–	–	–	–	–	–	1.00
95	–	1.00	0.14	–	1.00	–	–	–	–	–
90	–	–	–	–	–	1.00	–	–	–	–
Got-1										
106	0.02	0.08	–	0.02	0.04	–	–	–	–	0.06
102	–	–	–	–	–	–	0.15	–	–	–
100	0.98	0.92	1.00	0.98	0.96	1.00	–	–	–	–
97	–	–	–	–	–	–	0.85	1.00	1.00	0.94
Got-2										
105	–	–	–	–	–	–	–	–	–	1.00
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–
Hk-1										
104	–	0.02	–	–	–	–	0.02	–	1.00	1.00
100	1.00	0.98	1.00	1.00	1.00	1.00	0.98	1.00	–	–
Hk-2										
105	–	–	–	–	–	–	–	–	1.00	1.00
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–

Locus	SYL	FLA	ALP	HER	URA	HYR	MYS	EPI	PEN	AGR
Ck										
105	–	–	–	–	–	–	0.01	–	–	–
100	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00
Adk										
103	0.01	–	–	–	–	–	–	–	–	–
100	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pgm-1										
110	0.02	–	–	–	–	–	–	–	–	
103	0.02	0.03	–	–	–	–	0.04	1.00	–	
100	0.94	0.96	1.00	0.99	1.00	1.00	0.96	–	–	
95	0.02	0.01	–	0.01	–	–	–	–	0.94	
90	–	–	–	–	–	–	–	–	0.06	
Pgm-2										
104	0.01	0.04	–	–	0.01	–	0.04	–	–	–
100	0.99	0.96	1.00	1.00	0.99	1.00	0.96	1.00	1.00	0.77
90	–	–	–	–	–	–	–	–	–	0.23
Est-1										
106	–	–	–	–	–	–	–	–	1.00	0.61
103	–	–	–	0.04	0.11	–	0.06	–	–	–
100	1.00	1.00	1.00	0.96	0.87	1.00	0.88	–	–	0.39
95	–	–	–	–	0.02	–	0.06	1.00	–	–
Est-2										
106	–	–	–	–	–	–	–	–	1.00	–
104	–	–	–	–	–	–	0.06	1.00	–	–
100	1.00	0.98	0.91	0.81	–	1.00	0.77	–	–	1.00
98	–	–	–	–	0.17	–	0.17	–	–	–
95	–	0.02	0.09	0.19	0.83	–	–	–	–	–
Est-3										
110	–	–	–	–	0.01	0.06	0.02	–	–	–
108	–	–	–	–	–	–	–	–	–	1.00
105	0.04	0.01	–	–	–	–	0.82	1.00	1.00	–
100	0.87	0.68	1.00	0.99	0.99	0.94	0.16	–	–	–
95	0.09	0.31	–	0.01	–	–	–	–	–	–
Acph										
105	–	–	–	–	–	0.04	1.00			
100	1.00	1.00	1.00	1.00	1.00	0.96	–			
Pep-1										
109	–	–	–	–	0.01	–	–	–	–	–
105	–	–	–	0.02	0.99	1.00	1.00	–	1.00	–
100	1.00	0.27	–	0.98	–	–	–	1.00	–	1.00
94	–	0.73	1.00	–	–	–	–	–	–	–
Pep-2										
106	–	–	–	–	–	–	–	–	1.00	–
103	–	–	–	–	–	–	–	1.00	–	–
100	1.00	1.00	1.00	–	0.99	1.00	–	–	–	–
96	–	–	–	1.00	–	–	–	–	–	1.00
90	–	–	–	–	0.01	–	1.00	–	–	–
Pep-3										
100	1.00	0.95	1.00	1.00	0.99	1.00	–	–		
97	–	0.05	–	–	0.01	–	1.00	1.00		
Ada										
125	–	–	–	–	–	–	–	–	–	1.00
120	–	–	–	–	–	–	0.05	–	–	–
115	–	–	–	0.07	–	–	–	–	–	–

113	–	–	–	–	–	0.19	0.93	1.00	1.00	–
108	0.05	0.07	0.08	0.07	0.10	–	–	–	–	–
105	–	–	–	–	–	0.82	–	–	–	–
100	0.91	0.90	0.92	0.86	0.88	–	–	–	–	–
95	0.02	0.02	–	–	–	–	–	–	–	–
90	0.01	–	–	–	–	–	0.02	–	–	–
85	0.01	0.01	–	–	0.02	–	–	–	–	–
Fum										
103	–	–	–	–	–	–	–	–	1.00	1.00
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–
Aldo										
105	–	–	–	–	–	–	–	–	1.00	–
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–
95	–	–	–	–	–	–	–	–	–	1.00
Mpi										
103	0.01	0.02	–	–	–	–	–	–	–	–
100	0.97	0.98	1.00	1.00	0.99	1.00	0.14	–	–	–
97	–	–	–	–	–	–	0.86	–	–	–
95	0.02	–	–	–	0.01	–	–	–	0.25	–
93	–	–	–	–	–	–	–	1.00	–	–
90	–	–	–	–	–	–	–	–	0.75	1.00
Pgi										
104	–	0.01	–	–	0.01	–	–	–	–	–
100	0.99	0.91	1.00	0.73	0.96	1.00	1.00	1.00	1.00	–
96	0.01	0.06	–	0.27	0.03	–	–	–	–	–
94	–	–	–	–	–	–	–	–	–	1.00
90	–	0.02	–	–	–	–	–	–	–	–