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Biogeography and taxonomy of *Apodemus sylvaticus* (the woodmouse) in the Tyrrhenian region: enzymatic variations and mitochondrial DNA restriction pattern analysis

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In the western Mediterranean area, the taxonomic status of the various forms of *Apodemus sylvaticus* is quite unclear. Moreover, though anthropogenic, the origins of the island populations remain unknown in geographical terms. In order to examine the level of genetic relatedness of insular and continental woodmice, 258 animals were caught in 24 localities distributed in Belgium, France, mainland Italy, Sardinia, Corsica and Elba. Electrophoresis of 33 allozymes and mtDNA restriction fragments were performed and a UPGMA dendrogram built from the indices of genetic divergence. The dendrogram based on restriction patterns shows two main groups: 'Tyrrhenian', comprising all the Italian and Corsican animals and 'North-western', corresponding to all the other mice trapped from the Pyrenees to Belgium. Since all the Tyrrhenian mice are similar and well isolated from their relatives living on the western edge of the Alpine chain, they must share a common origin. The insular populations are consequently derived from peninsular Italian ones. From a taxonomic point of view and taking the priority rules into account, we have to invalidate *A. s. clanceyi* Harrison, 1948 and to consider the Tyrrhenian woodmice as belonging to *A. s. milleri* de Beaux, 1926, whereas the North-western ones must be referred to as the nominal subspecies. As far as the Elban woodmouse is concerned, at the moment we prefer to keep its present subspecific status because we only studied one animal.

Keywords: allozymes, *Apodemus sylvaticus,* biogeography, Italy, mitochondrial DNA, taxonomy.

Introduction

Despite several morphological studies (Kahmann, 1969; Pasquier, 1974; Darviche, 1978; Filippucci *et al.*, 1984; Alcover & Gosalbez, 1988; Kowalski & Rzebik-Kowalska, 1991; Libois *et al.*, 1993; Sara & Casamento, 1995) and a general revision of the genus *Apodemus* throughout France (Saint Girons, 1966, 1967), the subspecific status of the various Mediterranean woodmouse populations remains unclear.

In continental western Europe, four subspecies are currently considered (Toschi & Lanza, 1965; Saint Girons, 1973). *Apodemus sylvaticus sylvaticus* (L., 1758) is the most widespread in an area delimited by the Pyrenees and the Alps and whose northern limit reaches Scandinavia and the British Isles (mainland Ireland and U.K.) dichrurus (Rafinesque, 1814) is viewed as the 'Mediterranean' subspecies inhabiting Sicily (*Locus typicus*), Sardinia, Corsica, southern Italy, southern France and Spain. The range of *A. s. callipides* (Cabrera, 1907) includes the Cantabric and the Pyrenean chains and extends towards the Massif Central. *milleri* de Beaux, 1926 (= *clanceyi* Harrison, 1948) is restricted to northern and central Italy. Moreover, numerous insular populations have received subspecific status because of their large size or cranial differences, for example in

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the Hebrides and Shetlands (Berry *et al.*, 1967; Berry, 1973; Flowerdew, 1991) but also in the Mediterranean area: *A. s. hermani* (Felten & Storch, 1970) on the Pantelleria island, *A. s. ilvanus* Kahmann & Niethammer, 1971 on Elba, *A. s. eivissensis* (Alcover & Gosalbez, 1988) on Ibiza.

The boundaries of the geographical areas of the mainland subspecies are still confusing and cannot be interpreted easily because they do not correspond to any obvious geographical barrier. For example, in the eastern Pyrenees, it is quite impossible to draw any ecological or geographical limit between the two forms supposed to live there: in suitable habitats, the woodmouse is present everywhere, from the coast to the subalpine bushes (Fons *et al.*, 1980; Libois *et al.*, 1983). Moreover, the woodmice of Elba, Sardinia and Sicily were found to be genetically very close to those of peninsular Italy (Filippucci, 1987, 1992) although the populations of Sicily were considered by Von Lehmann & Schaefer (1976) to be very different and were assigned to a separate species, *A. dichrurus* Rafinesque, 1814. Finally, there is archaezoological evidence that the woodmouse was not present in Corsica and Sardinia until the beginning of the third millennium BC and the anthropogenic origin of its presence is taken for granted (Sanges & Alcover, 1980; Vigne, 1983).

The origins of the woodmouse populations on the different islands are important to determine if we are to achieve a better understanding of the biogeography of the species in the western Mediterranean basin. In this article, we report an overall genetic comparison of different woodmice populations living in that area. In *Mus musculus domesticus* (the house mouse), very important karyotypic differences are observed, even in restricted geographical areas (Said & Britton-Davidian, 1991; Searle, 1991; Mathias, 1992). No such differences have ever been discovered among *A. sylvaticus* populations. Moreover, the karyotype and the G and Q-banding patterns of the woodmouse are very similar to those of *A. flavicollis* (the yellow-necked mouse) (Engel *et al.*, 1973). However, Giagia (1985) reports some rare individuals bearing supernumerary acrocentric chromosomes whose significance remains unclear, their occurrence being higher in polluted areas (caused by heavy metals and chemicals). Consequently, karyotypic techniques do not appear well suited to our study. Two molecular techniques were thus applied: an electrophoretic analysis of allozymes and a study of mtDNA variation. The first technique allowed us to compare our results with those of Filippucci (1987, 1992) whereas the second, which is far more sensitive because of the fast evolutionary rate of mtDNA (Brown, 1980; Ferris *et al.*, 1983a; Wilson *et al.*, 1985; Moritz *et al.*, 1987), gave us data we could compare with those of Tegelström & Jaarola (1989) and Van Rompaey (1989).

Materials and methods

Biochemical and mitochondrial DNA analyses were performed on specimens representing a total of 24 wild populations from Belgium, France, Italy and also three Mediterranean islands (Sardinia, Corsica and Elba). The animals were trapped alive with 'Manufrance' traps. The sampling localities are presented in Table 1 and Fig. 1.

Electrophoretic analysis of allozyme variation

Electrophoresis was carried out using tissues from 191 animals (Table 1). Tissues were maintained at −80 °C until processed. Homogenates were obtained from pieces of muscle tissue for electrophoresis and screened for the following alloenzymes using the methods of Filippucci *et al.* (1984) and Filippucci (1992): α-glycerophosphate dehydrogenase (EC 1.1.1.8; *α-Gpdh*), lactate dehydrogenase (EC 1.1.1.27; *Ldh-1* and *Ldh-2*), malate dehydrogenase (EC 1.1.1.37; *Mdh-1* and *Mdh-2*), malic enzyme (EC 1.1.1.40; *Me-1* and *Me-2*), isocitrate dehydrogenase (EC 1.1.1.42; *Idh-1* and *Idh-2*), 6-phosphogluconate dehydrogenase (EC 1.1.1.44; *6-Pgdh*), glucose-6-phosphate dehydrogenase (EC 1.1.1.49; *G6pdh*), glyceraldehyde-3-phosphate dehydrogenase (EC 1.1.212; *G3pdh*), indophenol oxidase (EC 1.15.1.1; *Ipo-1* and *Ipo-2*), nucleoside phosphorylase (EC 2.4.2.1; *Np*), glutamic-oxaloacetic transaminase (EC 2.6.1.1; *Got-1* and *Got-2*), hexokinase (EC 2.7.1.1; *Hk-1* and *Hk-2*), creatine kinase (EC 2.7.3.2; *Ck*), adenylate kinase (EC 2.7.4.3; *Adk*), esterase (EC 3.1.1.1; *Est-3*), acid phosphatase (EC 3.1.3.2; *Acph*), leucyl aminopeptidase (EC 3.4.11.1; *Lap*), aminopeptidase (EC 3.4.11.11; *Ap-2* and *Ap-3*) adenosine deaminase (EC 3.5.4.4; *Ada*), aldolase (EC 4.1.2.13; *Aldo*), fumarate hydratase (EC 4.2.1.2; *Fum*), mannose-6-phosphate isomerase (EC 5.3.1.8; *Mpi*), glucose-6-phosphate isomerase (EC 5.3.1.9; *Gpi*), phosphoglucomutase (EC 5.4.2.2; *Pgm-1* and *Pgm-2*).

Isozymes were numbered in order of decreasing mobility from the most anodal band. Allozymes were numbered according to their mobility, relative to the most common allele (= 100) in the population of Burano (Nascetti & Filippucci, 1984) (< 100 = slower mobility; > 100 = faster mobility). Allozymic data were analysed as allele frequencies

Table 1 Sampling localities and number of *Apodemus sylvaticus* processed by each technique

Locality	Total no. of animals	No. of animals (allozyme analysis)	No. of animals (mtDNA analysis)	Sample symbols (see Fig. 1)
Belgium				
Sart Tilman	9	9	6	B1
Héron	3	0	3	B2
Seilles	5	0	5	B3
Namur	8	3	8	B4
Italy				
Tarquinia, Latium	31	21	31	I1
Grosseto, Tuscany	4	4	4	I2
San Polo dei cavalieri, Latium	6	6	0	I3
Burano lake, Tuscany	91	91	0	I4
Gambarie, Calabria	5	0	5	I5
Cuneo, Piedmont	1	0	1	I6
Sardinia				
Pietru	6	1	6	S1
San Antonio	1	0	1	S2
P. Tricoli	7	7	0	S3
Elba				
Monte Perone	13	12	1	E1
Corsica				
Fango (river)	10	10	10	C1
Fango (mouth)	7	7	7	C2
Chiuni	1	1	1	C3
France				
Cap Lardier	7	7	7	F1
Banyuls/Mer	12	12	6	F2
Massane	8	0	8	F3
Mont Vinaigre	4	0	4	F4
La Penne	6	0	6	F5
St Brisson	11	0	11	F6
Menigoute	2	0	2	F7

with the BIOSYS-1 program of Swofford & Selander (1981). Intrapopulational genetic variation was estimated by the mean heterozygosity per locus (H_e = expected frequency of heterozygotes under a Hardy–Weinberg equilibrium; Nei, 1978), proportion of polymorphic loci in the population ($P_{0.01}$, i.e. a locus is considered as polymorphic if the frequency of the common allele is not greater than 0.99), and the mean number of alleles per locus (A). The level of genetic divergence between populations was estimated using the indices of standard genetic identity (I) and distance (D) proposed by Nei (1978).

Mitochondrial DNA restriction pattern analysis

Mitochondria were isolated from fresh or frozen (liquid N_2) liver, heart, spleen and kidney according to Lansman *et al.* (1981). mtDNA was isolated using the method of Palva & Palva (1985) and then digested with restriction enzymes. In each case, 1–3 μL mtDNA solution containing 20–40 ng DNA were used. Two types of tetranucleotide restriction endonucleases (Boehringer Mannheim or BRL) were utilized: *Hae*III (GGCC) and *Rsa*I (GTAC). Digests were carried out for 1–2 h in 10 μL reaction liquid containing one enzyme unit. The mtDNA fragments were separated in 4 per cent PAA gels according to Tegelström (1986) and revealed by the silver staining protocol of Guillemette & Lewis (1983). All distinctive mtDNA restriction fragment patterns produced by *Hae*III were assigned a letter. Restriction fragment patterns produced by *Rsa*I were assigned a number. Each animal was thus assigned a letter and a number. All specimens

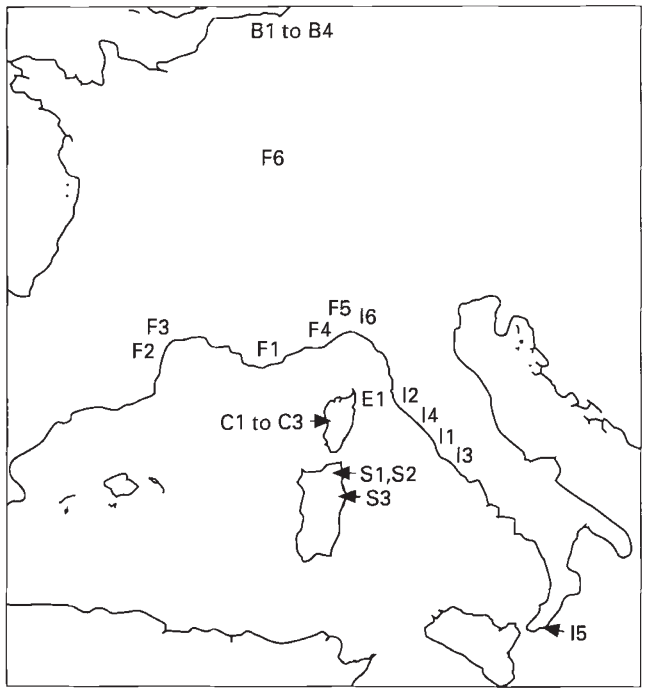

Fig. 1 Geographical distribution of the samples of *Apodemus sylvaticus*.

sharing a common composite restriction pattern were considered to belong to the same mtDNA matrilineal clone.

The proportion of shared fragments between two individuals was calculated with Dice's index as:

$$F = 2N_{xy}/(N_x + N_y),$$

where N_x and N_y are the numbers of fragments in individuals X and Y, and N_{xy} is the number of fragments shared by X and Y. Values of Dice's index were converted to estimate the nucleotide sequence divergence, p, according to Nei & Li (1979). Dendrograms were constructed from matrices of p-values by the unweighted pair group method (UPGMA; Sokal & Sneath, 1963).

Results

Electrophoretic analysis of allozyme variation

A total of 191 animals representing 14 populations have been studied electrophoretically. Fourteen of 33 loci were monomorphic and fixed for the same allele in all of the 14 populations analysed: *Ldh-1*, *Mdh-1*, *Mdh-2*, *Idh-2*, *G6pdh*, *G3pdh*, *Ipo-2*, *Hk-1*, *Ck*, *Ap-2*, *Ap-3*, *Lap*, *Acph* and *Aldo*. The other loci were polymorphic.

Four alleles were characteristic of some Corsican animals: *Ldh-2* (92), *Adk* (90), *Fum* (96) and *Got-2* (92); the last was also found in the sample from Elba. In spite of these peculiarities, these mice remain very close to the peninsular Italian ones. Indeed, they share with the latter, the *Np* (104) allele which has been found exclusively among the Italian woodmice.

Levels of genetic variation within samples are given in Table 2. The overall mean observed heterozygosity (H_o) was 0.026 with values ranging from 0.016 (Sardinia) to 0.052 (Namur). The overall mean proportion of polymorphic loci ($P_{0.01}$) was 15.6 per cent, ranging from 6.2 (Sardinia) to 34.4 per cent (Corsica). The overall mean proportion of alleles per locus (A) was 1.19, with values varying between 1.1 and 1.4. These values are consistent with those observed in other populations of the genus *Apodemus* (Benmehdi *et al.*, 1980; Gemmeke, 1980; Mezhzherin, 1990; Britton-Davidian *et al.*, 1991; Filippucci, 1992). They illustrate a great homogeneity in all the populations under study. Moreover, they belong to the range generally reported for other species of rodent (Nevo *et al.*, 1990).

Nei's (1978) values of genetic identity (I) and distance (D) were calculated amongst samples for all pair-wise comparisons (Table 3). The mean genetic distance between the samples was 0.0033, with values ranging from 0.00–0.015. Our results are similar to those of other studies on Mediterranean *A. sylvaticus* populations (Benmehdi *et al.*, 1980; Filippucci, 1987, 1992). Very small genetic distances such as these render any interpretation extremely tentative.

Table 2 Levels of genetic variation, based on 33 loci, in different populations of *Apodemus sylvaticus*

Samples	No. of individuals	$P_{0.01}$	H_o	H_e	A
F1	7	12.5	0.018	0.025	1.1
F2	12	28.1	0.029	0.028	1.3
B1	9	9.4	0.018	0.028	1.1
B4	3	9.4	0.052	0.040	1.1
C1, C2, C3	18	34.4	0.038	0.059	1.4
S1, S3	8	6.2	0.016	0.014	1.1
E1	13	12.5	0.026	0.027	1.2
I1	21	25	0.030	0.032	1.3
I4	91	25	0.020	0.024	1.4
I2	4	15.6	0.039	0.048	1.2
I3	6	9.4	0.031	0.028	1.1

$P_{0.01}$ = proportion of polymorphic loci using a 1% criterion; H_o = observed heterozygosity; H_3 = expected heterozygosity; A = average number of alleles per locus.

Table 3 Values of Nei's (1978) genetic identity (*I*, above the diagonal) and distance (*D*, below the diagonal) between the samples of *Apodemus sylvaticus*

Samples	F1	F2	B1	B4	C1, C2, C3	S1, S3	E1	I1	I4	I2	I3
F1	—	1.000	1.000	1.000	0.996	0.998	0.997	1.000	0.998	0.997	1.000
F2	0.000	—	0.999	0.996	0.996	0.999	0.998	0.999	1.000	0.998	0.999
B1	0.000	0.001	—	1.000	0.996	0.997	0.998	1.000	0.997	1.000	1.000
B4	0.000	0.004	0.000	—	0.994	0.993	0.993	0.999	0.994	0.998	1.000
C1, C2, C3	0.004	0.004	0.004	0.006	—	0.998	0.999	0.997	0.996	0.998	0.996
S1, S3	0.002	0.001	0.003	0.007	0.002	—	1.000	0.998	0.999	0.997	0.998
E1	0.003	0.002	0.002	0.007	0.001	0.000	—	0.998	0.998	1.000	0.998
I1	0.000	0.001	0.000	0.001	0.003	0.002	0.002	—	0.998	0.999	1.000
I4	0.002	0.000	0.003	0.006	0.004	0.001	0.002	0.002	—	0.998	0.998
I2	0.003	0.002	0.000	0.002	0.002	0.003	0.000	0.001	0.002	—	1.000
I3	0.000	0.001	0.000	0.000	0.004	0.002	0.002	0.000	0.002	0.000	—

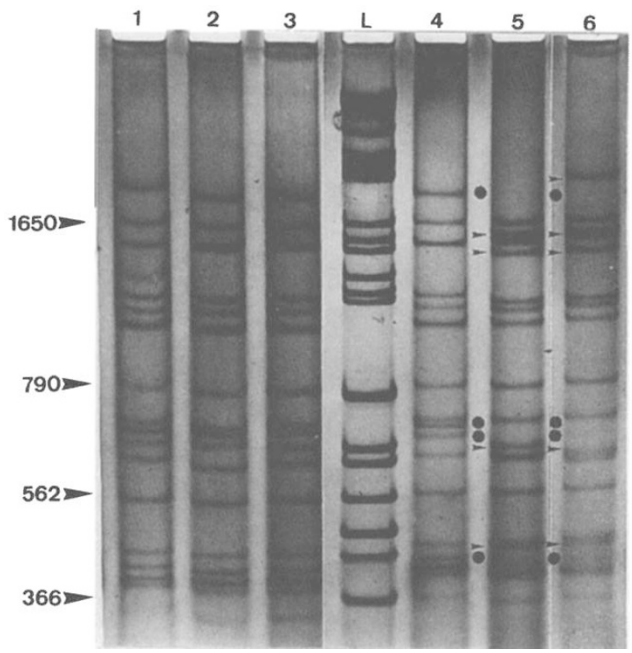

Fig. 2 Representative examples of fragment patterns after *Hae*III restriction endonuclease digestion of *Apodemus sylvaticus* mtDNA from: 1, S1; 2, I1; 3, E1; 4, C1; 5, F3; 6, B3. The lane marked L contains Lambda DNA digested with restriction endonuclease *Bgl*I to produce fragment size markers, the sizes of which are indicated on the left. The fragment sizes (in bp) of the four Tyrrhenian animals are: 2000, 1700, 1475, 1475, 1200, 1115, 1050, 840, 740, 730, 715, 640, 590, 480, 450, 410, 400 and 370. The dots show the fragments which are lacking in the western European animals (F3 and B3), and the arrows indicate the fragments which are lacking in the Tyrrhenian ones.

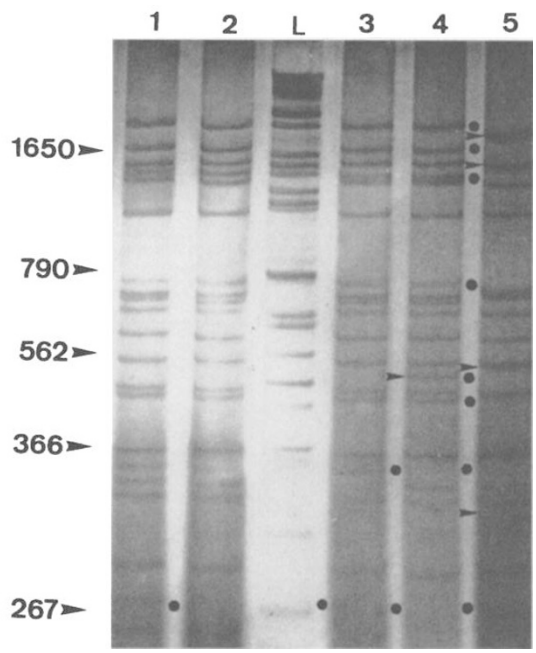

Fig. 3 Representative examples of fragment patterns after *Rsa*I restriction endonuclease digestion of *Apodemus sylvaticus* mtDNA from: 1, I1; 2, I1; 3, C1; 4, C2; 5, F3. The lane marked L contains Lambda DNA digested with restriction endonuclease *Bgl* I to produce fragment size markers, the sizes of which are indicated at the left. The fragment sizes (in bp) of the reference animal (I1 in lane 1) are: 1925, 1700, 1425, 1220, 1180, 1000, 730, 700, 700, 670, 590, 530, 475, 455, 350, 315, 300, 285, 235, 235, 215, 195, 140 and 135. Comparing the other lanes with the reference animal, a dot appears in front of a fragment which is lacking and an arrow in front of a new one.

mtDNA restriction pattern analysis

A total of 133 animals representing 21 populations have been analysed. Using two restriction endonucleases, we obtained a total of 42–46 mtDNA fragments for each animal. This number of restriction fragments seems quite adequate to obtain a good estimation of the divergence amongst the samples as far as nucleotide sequences are concerned (Ferris *et*

al., 1983a,b). The tables of the different fragment sizes obtained after digestion by *Hae*III and *Rsa*I are available upon request from the first author.

We did not observe obvious differences in the total length of the mtDNA as recalculated after interpretation of each restriction pattern, a potential problem raised by Tegelström & Jaarola (1989). We obtained 57 different clones from the animals examined. Some of the most representative are illustrated in Figs 2 and 3. The greatest sequence divergence

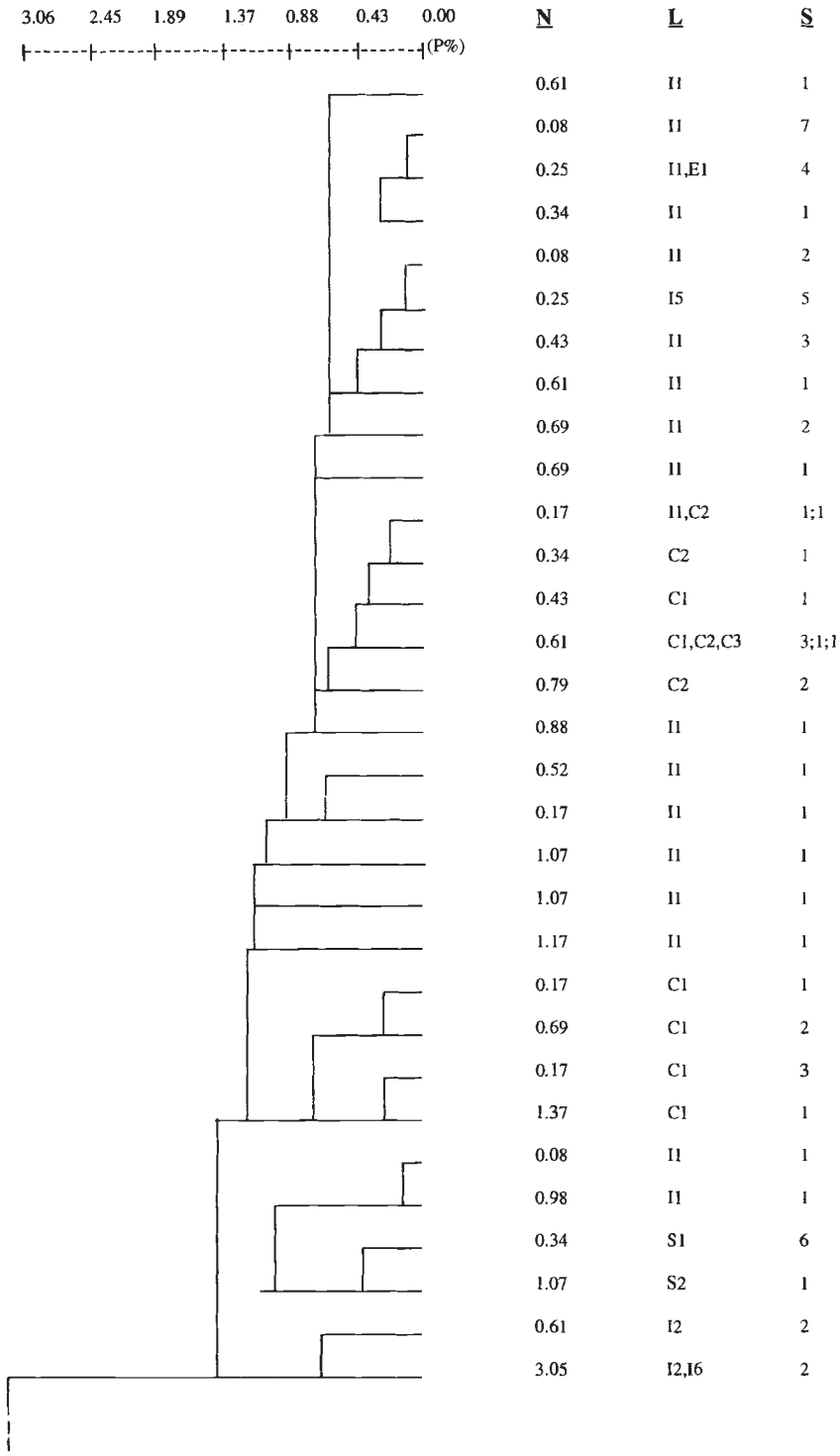

Fig. 4 UPGMA cluster analysis dendrogram of the 57 mtDNA-identified clones. Nucleotide sequence divergence is calculated according to Nei & Li (1979). N, level of the node in nucleotide sequence divergence units; L, locality (see Table 1); S, sample.

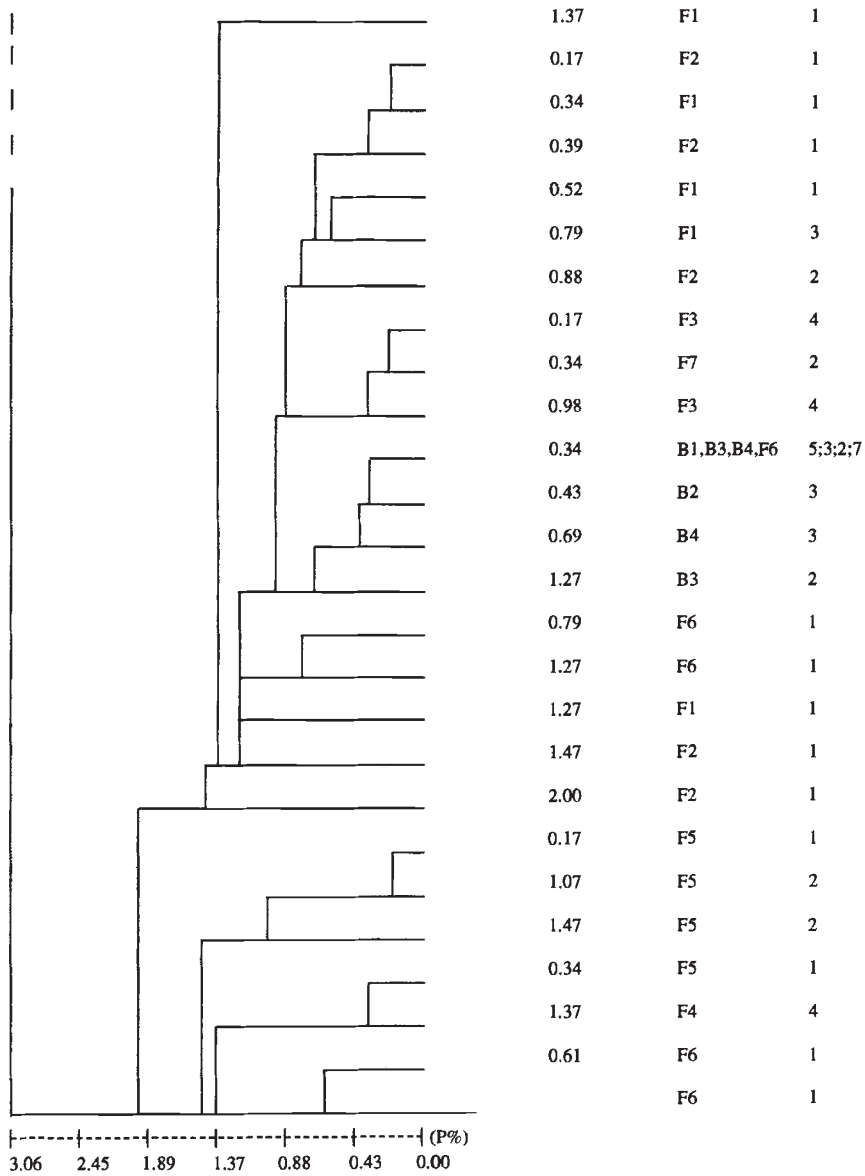

Fig. 4 *continued.*

found between the clones was 4.50 per cent ($F = 0.59$). The UPGMA dendrogram (Fig. 4) shows that these clones were well separated into two very distinct clusters. The first cluster collected all the Tyrrhenian animals, i.e. those from peninsular Italy including the Piedmont and from the three islands, Elba, Sardinia and Corsica. The second one included all the animals trapped in Belgium and in different regions of France, including the Pyrenees and the western Alps. The mean level of the sequence divergence of nucleotides between these two groups is quite high: $p = 3.05$ per cent whereas the intragroup level is very low: 0.36 and 0.79 per cent, respectively, in the Tyrrhenian and in the French groups. The latter values are of the same order of magnitude as those observed using the same technique in woodmouse populations of northern Europe ($p < 1$ per cent) (Tegelström & Jaarola, 1989; Van Rompaey, 1989) and in other rodent species (Brown & Simpson, 1981; Ferris *et al.*, 1983a,b).

Discussion

The enzymatic analysis shows a great homogeneity between all the populations studied, except for some

animals from Corsica and Elba which have some particular alleles. This technique does not reveal sufficient variation upon which to draw conclusions. On the contrary, the variation observed in the mtDNA restriction patterns allows inferences to be made on the following points.

The existence of at least two woodmouse groups in the western Mediterranean basin

The mean level of sequence divergence of nucleotides in the 'French' group is of the same order of magnitude as the value observed in the British Isles and in northern Europe including Belgium ($p < 1$ per cent) (Tegelström & Jaarola, 1989; Van Rompaey, 1989). We can thus assume that our 'French' group is part of a bigger 'North-western' group corresponding to the nominal subspecies of *A. sylvaticus* and extending towards Scandinavia. As we trapped many animals in the Mediterranean (Cap Lardier, Banyuls/Mer) and in the Pyrenees (Massane) biomes, we can no longer accept the opinion of Saint Girons (1973) about the presence of *A. sylvaticus dichrurus* (Rafinesque, 1814) in southern France and of *A. sylvaticus callipides* (Cabrera, 1907) in the Pyrenean region. She is also probably wrong in writing that *A. sylvaticus clanceyi* has been observed in the French southern Alps.

The great homogeneity all over continental western Europe has to be checked in the Iberian peninsula. This could confirm a postglacial recolonization of western Europe from a refuge population which would have survived close to the pre-Pyrenean border of the Mediterranean sea or on the southern side of these mountains.

As far as the Tyrrhenian group is concerned, the level of nucleotide divergence from the North-western group is so high (>3 per cent) that a subspecific status of that group seems quite appropriate. Indeed, Ferris *et al.* (1983a,b) consider that a level of 4 per cent divergence could lead to the distinction of different subspecies. Bearing in mind the taxonomic laws of priority, the Tyrrhenian woodmouse should be named *A. sylvaticus milleri* de Beaux, 1926 and *A. sylvaticus clanceyi* Harrison, 1947 considered as synonym. In the same way, we could consider *A. sylvaticus ilvanus* Kahmann and Niethammer, 1971 also as a synonym. Indeed, gigantism appears to be a general response of small species exposed to insularity conditions, particularly on very small islands: e.g. *Apodemus* on Elba (Kahmann & Niethammer, 1971), on Pantelleria (Felten & Storch, 1970), Porquerolles (Libois & Fons, 1990) and Ibiza (Alcover & Gosalbez, 1988) and *Crocidura suaveolens* on Corsica, Yeu, Ouessant and Sein (Saint Girons, 1973).

Nevertheless, considering that (i) our mtDNA analysis was made on just one specimen, (ii) the morphological differentiation of this population is particularly important and (iii) morphological and molecular evolution appear to proceed independently in mammals (Schnell & Selander, 1981), we have to be careful on this point.

Our interpretation agrees with the results of Gemmeke *et al.* (1987) which emphasize the contrast in the transferin structure between woodmice living in Spain, France, Switzerland, Germany and even Tunisia on one hand and those living in Italy, Sardinia, eastern Austria and Slovenia on the other.

The Alps as a biogeographical barrier

The Alps are well known as a biogeographical barrier for many animals, for example the small mammal species *Talpa caeca*, *Crocidura leucodon*, *Pitymys savii*, *Pitymys duodecimcostatus*, *Clethrionomys glareolus*, *Arvicola sapidus* and *Mus spretus*, several bird species such as *Dryocopus martius* (the black woodpecker), *Phylloscopus trochilus* (the willow warbler), *Sylvia curruca* (the lesser whitethroat) and *Passer domesticus italiae* (the Italian house sparrow), and for 20 species of reptiles and amphibians (Orsini, 1990). For ground dwelling species, the obstacle is obvious: high summits with glaciers in the north, and dry, bare hills falling directly into the sea on the western edge. This barrier also appears to be very efficient for the highly adaptable woodmouse. Indeed, all the animals trapped on the western edge of the Alps (Estérel, La Penne) display a clear North-western mtDNA restriction pattern whereas that of the Piedmont (Cuneo) displays a typical Tyrrhenian one. This raises the question of exactly when these two woodmouse strains parted from each other.

From the enzymatic study, the separation could be about 15 000 years old, assuming a rate of change of one distance unit per 0.7–1.5 Myr (an estimate usually employed in protein-clock calibration for small mammals: Tegelström & Jaarola, 1989; Avise & Aquadro, 1982), but this is likely to be very inaccurate given the low values of *D*. The mtDNA study leads, however, to longer time estimates. Assuming a 2–4 per cent rate of mtDNA-sequence divergence/myr (Wilson *et al.*, 1985), the divergence between the Tyrrhenian and the North-western groups would be 730 000–1 460 000 years old. To explain similar discrepancies between the two evaluation methods, Tegelström & Jaarola (1989) and Van Rompaey

(1989) formulated the following hypotheses: (i) the rate of protein evolution is much slower than usually postulated, (ii) the rate of mtDNA divergence is much faster than previously assumed, (iii) mtDNA divergence predates nuclear divergence and (iv) there is nuclear gene flow between populations after the separation event.

These four hypotheses are not mutually exclusive. However, Van Rompaey's (1989) opinion is that the first two hypotheses are probably more appropriate to explain such differences: the calibration rate could be a major source of error and the 'technique' is still strongly debated (Nei, 1987). In this way, Catzeflis *et al.* (1992) have discovered that the calibration rate of the mtDNA molecular clock could be different between one mammal family and another. For example, the mtDNA of muroid rodents could evolve at least three times faster than that of primates and other mammals. Catzeflis *et al.* (1987) concluded that 'There is not a single, global DNA clock ticking at the same average rate in all mammals; rather, the rate of genomic evolution in each group must be determined separately by calibrating numbers of nucleotides changes with absolute divergence dates that are derived from fossils or vicariant events.

The origin of the woodmouse settlement in the Tyrrhenian islands

The Italian origin of the Tyrrhenian islands woodmice is taken for granted. As a result of the enzymatic analysis, we can suggest the route Etruria→Elba→Corsica as the most probable one. Having in mind the anthropogenic origin of these animals (Sanges & Alcover, 1980; Vigne, 1983), this hypothesis agrees with those of archaeologists (Klein Hoffmeiyer *et al.*, 1986) who observed the same type of human Neolithic culture in continental Italy, Elba and Corsica. That seems to indicate that there were some relationships between the Neolithic human populations established on these two islands and on peninsular Italy, offering colonization opportunities for the woodmouse.

The origin of the Sardinian woodmice is less obvious: archaezoologists (Cherry, 1990; Vigne, 1990) share the opinion that the colonization of Sardinia took place from Italy through Corsica. Our molecular results, however, show that animals of the two islands differ; the characteristic alleles of Corsican and Elban *Apodemus* are completely absent in Sardinian mice. Sardinia was thus probably invaded directly from Italy.

Conclusions

To sum up, the present study shows clearly that the taxonomy of the woodmouse on the western Mediterranean basin has to be reconsidered at the subspecific level.

1 From the Pyrenees to Belgium and even to Scandinavia and the mainland of the U.K. and Ireland (see Tegelström & Jaarola, 1989 & Van Rompaey, 1989), woodmice share very similar mtDNA restriction patterns. Consequently, they must belong to the same subspecies, nominally *A. s. sylvaticus.*

2 The populations of the Tyrrhenian islands and of peninsular Italy have a common origin and differ from the North-western subspecies. Archaezoological findings strongly suggest that the woodmouse was recently introduced to Corsica by man. The Sardinian colonization was also anthropogenic. Therefore, we can logically combine all these populations in the same subspecies, *A. s. milleri*, A. *s. clanceyi* becoming synonymous.

3 These Tyrrhenian mice are well isolated from those living on the western edge of the Alpine chain, either in the Mediterranean biome or in the more temperate zones including the eastern Pyrenean beech forest (Massane).

4 Until the validity of *A. s. callipides* is checked, we suggest that the extent of its geographical range in France is restricted to the western and possibly the central Pyrenees.

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