

SHORT NOTE

IS THE WOODMOUSE (*APODEMUS SYLVATICUS*) OF SICILY A DISTINCT SPECIES?

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The Sicilian population of *Apodemus sylvaticus* (L., 1758) was initially considered a separate subspecies *A. s. dichrurus* Rafinesque, 1814. Two «morphs», one occurring around Palermo and the other near the Etna (1) were subsequently designated as subspecies (2). The Sicilian woodmouse is now considered a distinct species, *A. dichrurus* Von Lehmann & Schaeffer, 1976, based on its higher alkaline phosphatase activity (3). To evaluate the specific status of *A. dichrurus*, we have now studied mtDNA variation amongst 85 individuals from various populations in the Western Mediterranean area (Fig. 1).

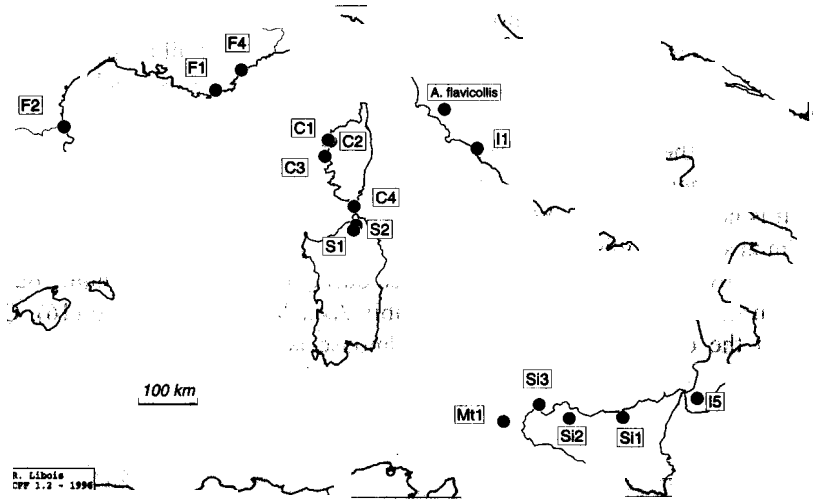


Fig. 1. - Geographic distribution of the sampling localities of *Apodemus*. F1: Cap Lardier*; F2: Banyuls/Mer*; F4: Estérel (Mt Vinaigre)*; I1: Tarquinia (Latium)*; I5: Gambarie (Calabria)*; C1: Fango*; C2: Fango (mouth)*; C3: Chiuni*; C4: Bonifacio; S1: Pietru*; S2: San Antonio*; Si1: Ficuzza; Si2: Grateri; Si3: St Vito lo Capo; Mt1: Marettimo; *A. flavicollis*: Grosseto (Tuscania). The localities with an * are the same as in Michaux *et al.* (1996) and are numbered in accordance.

Mitochondrial DNA was isolated from fresh tissue and digested with restriction endonucleases *Hae III* and *Rsa I*. The resulting fragments were separated in 4% PAA gels and subsequently silver stained (for further details: 4). The restriction patterns obtained were compared using the Nei & Li index (5) and a neighbour-joining tree (TREECON 1.2; see 6), was built using one individual yellow-necked field mouse *A. flavicollis* (Melchior, 1834) from Grosseto (Italy) as an outgroup. A bootstrap analysis (1000 replicates) was also performed to check the robustness of the nodes.

From the 86 animals analysed, 45 different haplotypes were obtained. The neighbour-joining tree (Fig. 2) shows that the yellow-necked field mouse haplotype is well separated from all the woodmice ones, the node separating them being very robust (BP of 100 %). The mean level of the nucleotide sequence divergence between the two species is 5.9%.

The woodmice are divided into three distinct clusters: one cluster contains all the animals from peninsular Italy, Corsica and Sardinia, a second one contains those trapped in France, and the third one is formed by all the Sicilian samples. The mean level of genetic divergence between these three groups is quite high (between 2.6 and 3.8%), and this separation is very well supported with bootstrap values of 92 and 97%. We can therefore consider that the Sicilian woodmice constitute a third mtDNA lineage. In contrast, the intra-group divergence is very low: 1.4, 0.9 and 1.2 respectively, and of the same order of magnitude as that observed using the same technique in woodmice populations of Northern Europe ($p \approx 1\%$) (4, 7, 8) and in other rodent species (9, 10, 11). On the other hand, the differences in the values observed between the three woodmice groups of haplotypes are similar to those differences observed between subspecies of *Mus domesticus* L. 1758 ($\approx 4\%$) (10, 11). From a morphological point of view, the Sicilian mice differ only slightly from those of Sardinia or of Peninsular Italy; these differences being less than those found between the Italian and the French populations (12, 13) which are not separated at a specific level. Furthermore, fertile hybrids between animals from Sicily and either Italy or Germany have been obtained in captivity (3). For all these reasons we propose retention of the distinction between the Sicilian woodmouse and the other west European woodmouse populations only at the subspecific level (*A. sylvaticus dichrurus*).

Nevertheless, the mtDNA differences suggest that the Sicilian woodmice became isolated from the other groups about 750 000 years ago (using the calibration of Wilson *et al.*: 14). It is now well established from archaeozoological data (15), that the presence of the woodmouse in Sicily is the consequence of a Holocene anthropogenic introduction. It is also well known that Sicily has been invaded during the Holocene by numerous human groups from different geographic areas, notably Asia Minor and Greece (16). Our data suggest that the origin of the Sicilian woodmouse is not Peninsular Italy or Western Europe. This seems rather surprising since the strait of Messina is only 3 km wide. Notwithstanding, it should be interesting to compare mtDNA variation between the Sicilian populations and those living in North Africa and in the eastern part of the Mediterranean basin.

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REFERENCES

1. HAGEN, B. (1954) – Zur Kleinsäugerfauna Siziliens. *Bonn. Zool. Beitr.*, **1-2**: 1-15.
2. VON LEHMANN, E. & H.E. SCHAEFFER (1973) – Untersuchungen von Waldmäusen (*Apodemus sylvaticus*) und Gelbhalsmäusen (*Apodemus flavicollis*) in Kalabrien und Sizilien. *Suppl. Ric. Biol. Selvaggina, Lab. Zool. appl. Caccia, Bologna*, **6**: 175-184.
3. VON LEHMANN, E. & H.E. SCHAEFFER (1976) – Kreuzungsergebnisse mit Waldmäusen (*Apodemus*) der Insel Sizilien., *Säugertierkd. Mitt.*, **24**: 180-184.
4. MICHAUX, J., M.-G. FILIPPUCCHI, R.M. LIBOIS, R. MATAGNE & R. FONS (1996) – Biogeography and taxonomy of the wood mouse (*Apodemus sylvaticus*) in the Tyrrhenian region: enzymatic variations and mitochondrial DNA restriction patterns analysis. *Heredity*, **76** (3): 267-277.
5. NEI, M. & W.-H. LI (1979) – Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA*, **76**: 5269-5273.
6. VAN DE PEER, Y. & R. DE WACHTER (1996) – Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Comput. Applic. Biosci.*, **13**: 227-230.
7. TEGELSTRÖM, H. & M. JAAROLA (1989) – Genetic divergence in mitochondrial DNA between the wood mouse (*Apodemus sylvaticus*) and the yellow necked mouse (*A. flavicollis*). *Hereditas*, **111**: 49-60.
8. VAN ROMPAEY, J. (1989) – *Variatie en differentiatie van mitochondrial DNA in Clethrionomys glareolus (Schreber, 1780), Apodemus sylvaticus (Linnaeus, 1758) en Apodemus flavicollis (Melchior, 1834) (Mammalia, Rodentia) in West Europa*. Ph.D Thesis. University of Antwerpen, (166 pp).
9. BROWN, G. & M. SIMPSON (1981) – Intra- and interspecific variation of the mitochondrial genome in *Rattus rattus* and *Rattus norvegicus*: restriction enzyme analysis of variant mitochondrial DNA molecules and their evolutionary relationships. *Genetics*, **97**: 125-143.
10. FERRIS, S.D., R.D. SAGE, C.M. HUANG, J.T. NIELSEN, U. RITTE, U. & A.C. WILSON, (1983a) – Flow of mitochondrial DNA across a species boundary. *Proc. Natl. Acad. Sci. USA*, **80**: 2290-2294.
11. FERRIS, S.D., R.D. SAGE, E.M. PRAGER., U. RITTE, & A.C WILSON (1983b) – Mitochondrial DNA evolution in mice. *Genetics*, **105**: 681-721.
12. SARA, M. & G. CASAMENTO (1996) – Morphometrics of the wood mouse (*Apodemus sylvaticus*, *Mammalia, Rodentia*) in the Mediterranean. *Boll. Zool.*, **62** (3): 313-320.
13. MICHAUX, J.R., R. LIBOIS & R. FONS (1996b) – Différenciation génétique et morphologique du mulot (*Apodemus sylvaticus*), dans le bassin méditerranéen occidental. *Vie et Milieu*, **46** (3/4): 193-203.
14. WILSON, A.C., R.L. CANN, S.M. CARR, M. GEORGE, U.B. GYLLENSTEIN, K.M. HELM-BYCHOWSKI, R.G. HIGUSHI, S.R., PALUMBI, E.M.M. PRAGER, R.D. SAGE & M. STONEKING (1985) – Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.*, **26**: 375-400.
15. TAGLIACCOZZO, A. (1993) – Archeozoologia della Grotta dell'Uzzo. *Suppl. Boll. Paleoetn. it.*, **84** (2): 1-278.
16. TUSA, S (1993) – La Sicilia nella Preistoria. (2nd edn). Palermo, Sicilia: Sellerio.

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