Italian Journal of Zoology

ISSN: 1125-0003 (Print) 1748-5851 (Online) Journal homepage: http://tandfonline.com/loi/tizo20

Microevolution in the sicilian shrew *crocidura sicula* (Mammalia, Soricidae) tested by RAPD-PCR fingerprinting

Maurizio Sarà , Cinzia Aiuto & Goffredo Cognetti

To cite this article: Maurizio Sarà , Cinzia Aiuto & Goffredo Cognetti (1997) Microevolution in the sicilian shrew *crocidura sicula* (Mammalia, Soricidae) tested by RAPD-PCR fingerprinting, Italian Journal of Zoology, 64:1, 41-48, DOI: 10.1080/11250009709356170

To link to this article: https://doi.org/10.1080/11250009709356170

Ital. J. Zool., 64: 41-48 (1997)

Microevolution in the Sicilian shrew *Crocidura sicula* (Mammalia, Soricidae) tested by RAPD-PCR fingerprinting

MAURIZIO SARÀ
CINZIA AIUTO
GOFFREDO COGNETTI
Dipartimento di Biologia Animale, Università di Palermo, via Archirafi 18, I-90123 Palermo (Italy)

ABSTRACT

Genetic variation in samples of the endemic *Crocidura sicula* living in Sicily and in two surrounding small islands, Marettimo and Ustica, was analysed by Random Amplified Polymorphic DNA fingerprinting (RAPD) and compared to morphometrics and external phenotypes. Molecular variation in the random sample of 99 DNA fragments of the Ustica shrews, showing a melanic fur and a size-shape variation in skull morphometrics, is of comparable size to that of the of northwestern and northeastern samples Sicily (Tufanio and Madonie). In the Marettimo shrews, bicoloured (grey and white) animals like those coming from Sicily and presenting a significant reduction in body-size and skull morphometrics, molecular differentiation is higher than in those of the other locations, and characterizes this geographic population. Considering the paleogeographic records, it is hypothesized that the Marettimo shrews must have been isolated longer from the mother-island; whereas for the Ustica shrews, a more recent arrival/isolations presumed. The RAPD results proved to be consistent with this reconstruction of the *C. sicula* biogeography. The Marettimo population, as opposed to that of Ustica, can thus be considered 'one step further' on the road of the speciation process. Melanism in the more recently isolated Ustica population does not parallel genetic differentiation and could emerge as an answer to the very strong selection and ecological adaptation in that volcanic insular environment. In any case, each population living in these two small islands off Sicily shows an independent evolutive divergence.

KEY WORDS: *Crocidura sicula* - Mediterranean endemism - RAPD-PCR - Morphometrics - Geographic variation.

ACKNOWLEDGEMENTS

This work was supported by a grant (MURST 60%, 1995) from the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica. The Authors thank Drs Cornelia Di Gaetano and Margherita Randazzo for their help in laboratory work, and Laura Zanca for help during field-trapping and for graphical support. Thanks are also due to the anonymous referees who gave helpful remarks on a previous draft.

(Received 15 June 1996 - Accepted 18 October 1996)

INTRODUCTION

The taxonomy of the genus *Crocidura*, a small insectivorous mammal (Soricidae), in continental and Mediterranean insular populations has been controversial for a long time. The recent application of karyological and biochemical techniques established the correct taxonomic position of the shrews living in Sardinia and Corsica (Catalan & Poitevin, 1981; Catzeflis, 1983; Vogel *et al.*, 1986). With regard to Sicily and its surrounding islands, Miller (1901) examined two specimens from Sicily and described them as two different species: *C. sicula*, with short tail and small skull, and *C. caudata* with a longer tail and larger skull. Since then, the taxonomic attribution of these shrews has been confused and disputed. Both the number of species present in the island and their taxonomic position have been matters of discussion. Miller's taxa were later considered either as true species (e.g. Vesmanis, 1976) or as subspecies of already known Paleoarctic species, and generally referred to as *C. leucodon sicula* (e.g. Wettstein, 1925) and *C. russula caudata* (e.g. Toschi, 1959). The taxon *sicula* was considered to be closely related (Vesmanis, 1976; Hutterer, 1981) also to a third species, *C. suaveolens*, which was considered to be present in the Egadi (Marettimo, Levanzo, Favignana) and Maltese (Malta, Gozo) archipelagos (Krapp, 1969; Schembri & Schembri, 1979).

Recent karyological studies (Vogel, 1988; Vogel *et al.*, 1989, 1990) gave a new and correct look at the previous interpretations, showing the presence of a new karyotype (2n = 36, NF = 56, NFa = 52) in the shrews living in Sicily and Gozo. Sarà *et al.* (1990) and Sarà (1995) showed by morphometrics that all these shrews, as well as the Egadi and Ustica taxa, are strictly related. Karyological analysis (Sarà & Vitturi, 1996) demonstrated that also the populations from Marettimo and Ustica have the same standard karyotype as *C. sicula*.

As a conclusion, the above data pointed to the existence of a good species, *Crocidura sicula*, which is endemic in the Sicilian-Maltese area, strictly insular, and probably a relict from the Pleistocene. This taxon, fragmented over several islands, also presents a noteworthy pattern of geographic variation in skull morphometrics, showing an independent divergence of each island's populations (Sarà, 1995).

This general pattern of geographic variation and the strikingly different phenotype of the Ustica shrews (Fig. 1) led us to investigate the presence and extent of microevolutive processes also from a biomolecular standpoint. We employed the Random Amplified Polymorphic DNA (RAPD-PCR) technique to have a preliminary insight into the molecular variability of these shrews, comparing Ustica and Marettimo samples to some individuals coming from northwestern and northeastern Sicily. Since its introduction by Williams *et al.* (1990), RAPD-PCR has been applied to a variety of problems in the field of molecular ecology. It has been used to deter-

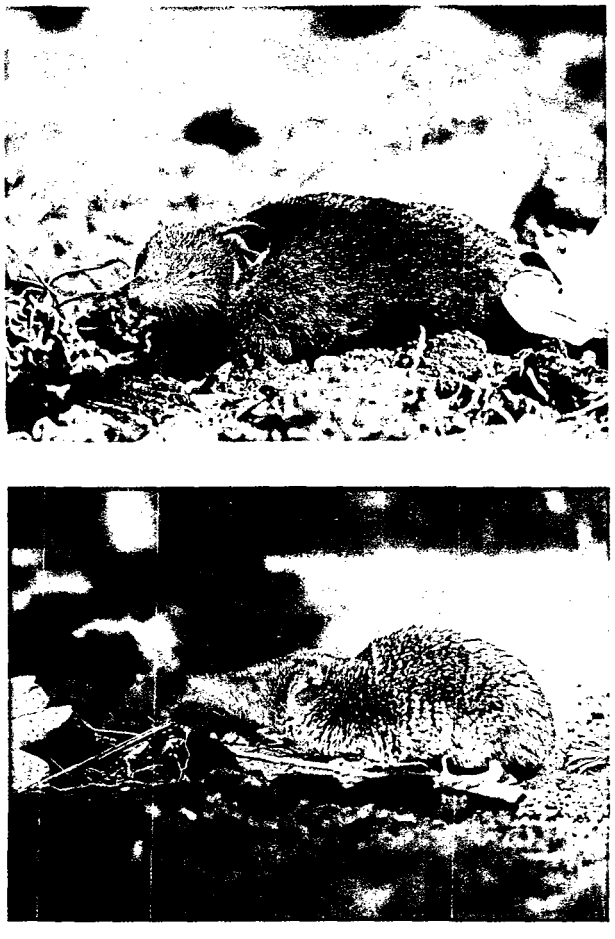

Fig. 1 - **A:** a Sicilian shrew (*Crocidura sicula*) from Sicily, Marettimo and Gozo, showing its bicoloured fur. **B:** a melanic *Crocidura sicula* from Ustica.

mine paternity and kinship (Hadrys *et al.*, 1993), gene flow (Arnold *et al.*, 1991), and taxonomic identity and/or taxonomic similarity in molecular evolution (reviewed by Hadrys *et al.*, 1992).

MATERIALS AND METHODS

Specimens

Twelve *Crocidura sicula* collected (three at Ustica, three at Marettimo and six in Sicily) during past (1990-92) live-trapping sessions or found dead in the field, were stored at the Zoological Museum of Palermo University. The main features of the locations, of the specimens, and of their storage conditions are reported in Table I and Figure 2. Some of these specimens had already been used for a previous karyological study (Sarà & Vitturi, 1996). Three of the mainland Sicilian specimens came from the northwestern (Tufanio near Alcamo - Province of Trapani) and the other three from the northeastern (Madonie - Province of Palermo) parts of the island.

DNA preparation

Genomic DNA was extracted from kidneys adapting the classical method (Herrmann & Frischauf, 1987), which involves crushing the tissue in liquid nitrogen, resuspension in sodium laurylsulfate/proteinase K, followed by phenol and chloroform extractions and isopropanol precipitation. RNA was eliminated by RNAse treatment.

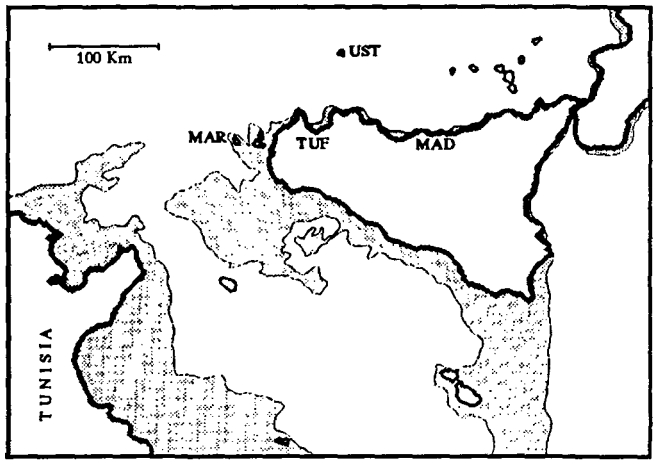

Fig. 2 - Map of Sicily and surrounding archipelagos, in which the sites of shrew collection are coded as: MAR, Marettimo; UST, Ustica; MAD, Madonie; TUF, Tufanio (cf. also Table I). Marettimo has 12 km^2 of surface area and a max. altitude of 696 m a.s.l.,whereas Ustica is 8 km^2 in surface area with a max. altitude of 238 m a.s.l. The stippled area shows the extension of land which emerged during the Late Pleistocene (Würmian), now forming a platform below sea level to a depth of 200 m.

RAPD-PCR

Random primers were purchased from Operon Technologies (Alameda, Ca.). Several primers were tested, and ten of them gave a pattern in all the specimens. Amplification conditions were those described by Williams *et al.* (1990) with the only difference that we employed the "Stoffel fragment" of Taq polymerase (Perkin-Elmer). Compared to normal Taq polymerase, Stoffel fragment is reported (Lawyer *et al.*, 1993) to be more thermostable, allowing therefore higher denaturation temperatures, and provided with a lower processivity. Such conditions facilitate electrophoretic separation and high reproducibility. Since the purity of water is critical, all the solutions were made in Milli Q-plus (Millipore) filtered water, and two blanks containing water instead of DNA were also tested in each amplification.

Electrophoresis

Small aliquots from the amplification products were run on 2% agarose gels in the presence of ethidium bromide, using the 100-bp marker ladder (Pharmacia). Amplification patterns were detected on a 312-nm UV screen and photographed. Enlarged prints were used for further calculations.

Computer analysis

The ten primers screened in the twelve specimens amplified a complex pattern of polymorphic genomic DNA. The mobility of the amplified fragments was measured connecting, with a ruler, the corresponding bands of the marker run in parallel at both sides of the gel. Each band in each specimen was scored as 1 if present (marker) or 0 if absent (null allele), according to Lynch & Milligan (1994). Letting M and m denote the marker and the 'null allele' at a locus and p and q their frequencies, the only observable quantity for a locus in a RAPD profile is the fraction of individuals in the population with (1-x) or without (x) the marker. Since the 'null allele' frequency is $q = x^{1/2}$, the resulting matrix of marker frequencies from single individuals will correspond to the gene frequency matrix. To avoid bias due to the small sample sizes, Lynch & Milligan (1994) suggest restricting the analysis to the uncommon bands, i.e. those with a frequency less than $1 - (3/n)$; where n = number of specimens. According to this rule, all the bands whose observed

TABLE I - *Origin of the twelve specimens of* Crocidura sicula *considered.*

No	Code	Location	Habitat	Altitude (m a.s.l.)	Karyotyped*	Tissue condition	DNA ng/µl
1	MAR	Marettimo (Egadi - TP)	Olive orchard	30	y	fresh, then -80° C	14.85
2	MAR	Marettimo (Egadi - TP)	Olive orchard	30	y	fresh, then -80° C	4.60
3	MAR	Marettimo (Egadi - TP)	Olive orchard	30	n	fresh, then -80° C	2.35
4	TUF	Tufanio (TP)	Olive orchard	50	y	fresh, then -80° C	20.65
5	TUF	Tufanio (TP)	Olive orchard	50	y	fresh, then -80° C	4.50
6	TUF	Tufanio (TP)	Olive orchard	50	n	fresh, then -80° C	2.10
7	UST	Ustica (PA)	Meadow	50	y	fresh, then -80° C	2.90
8	UST	Ustica (PA)	Meadow	50	y	fresh, then -80° C	11.05
9	UST	Ustica (PA)	Meadow	50	n	fresh, then -80° C	1.15
10	MAD	Piano Cervi (Madonie - PA)	Beech forest	1600	n	dead, then -20° C	0.75
11	MAD	Gratteri (Madonie - PA)	Oak wood	400	n	fresh, then -80° C	18.65
12	MAD	Campofelice (Madonie -PA)	Genista maquis	200	y	fresh, then -20° C	11.30

*, Sarà & Vitturi,1996.

frequency was ≥ 0.75 (i.e., present in 9-12 individuals) were discarded. The original binary matrix of 138 bands × 12 specimens was thus reduced to a second one of 99 bands × 12 specimens. This was then converted into a similarity one, employing the simple matching coefficient (SM), an association metric which takes into account both the number of matches (double presence, double absence) and of unmatches (absence in one sample and presence in a second; and vice-versa). This similarity matrix was treated by the unweighted *pair-group method algorithm* (UPGMA) to cluster the 12 specimens.

The analysis was then repeated considering the mean gene frequency of each sample of three specimens, according to the formula (Lynch & Milligan, 1994):

$$q = x^{1/2} \cdot \frac{[1 - \mathrm{Var}(x)]^{-1}}{8x^2}$$

where $\mathrm{Var}(x) = x \cdot (1-x)/n$, and $n = 3$ is the sampling variance of the frequency of null alleles. The terms in parenthesis account for much of the bias in estimation of q due to a small sample size. The mean gene frequency matrix was converted to a distance matrix, among the four samples, by the Nei (1972) genetic formula, and then clustered by UPGMA.

The conventional measure of gene diversity within a population, $Hj_{(0)} = 2qj_{(0)} \cdot [1-q_{(0)}]$, has also been drawn according to Lynch & Milligan (1994). This measure is equivalent to the expected heterozygosity under Hardy-Weinberg equilibrium and can be viewed as the probability that a random pair of alleles will contain one marker and one null. In spite of the mathematical corrections employed, the low number of specimens can result in a biased estimation of q and $Hj_{(0)}$, and thus these quantities can be viewed only as relative to this first exploratory study, and to the given geographic samples, and hence as not being representative of all the *C. sicula* populations.

There are several problems, both ethical and legal, in killing animals like shrews in order to have the larger samples necessary for a more robust analysis of population genetics. For these reasons we used only the few specimens already available at the Zoological Museum, without killing additional animals. These problems can be by-passed in further studies by using, as source of DNA, the large quantities of bones found in owl pellets (Taberlet & Fumagalli, 1996).

To compare genetic and morphometric variation, a canonical variate analysis of 14 metric variables measured over 74 skulls coming from the same localities (24 from northeastern Sicilian localities in the Madonie area; 17 from northwestern Sicilian localities in the Tufanio area; 20 from Marettimo; 13 from Ustica) was carried out. To measure the degree of relationship between morphometrics and genetic variation, a Mantel test was run on the two resulting distance matrices (Mahalanobis for morphometrics; Nei for genetics). The calculation of cophenetic correlations allowed us to evaluate the goodness of fit of each UPGMA clustering. All computer analyses were performed by the NTSYS-pc software (Rohlf, 1993).

RESULTS

PCR-amplification of shrew DNA by RAPD fingerprinting yielded a series of discrete fragments (Fig. 3). A total of 138 fragments was amplified by the 10 primers used in the study, but only 99 were retained for the analyses (Table II). Each primer gave a different RAPD profile amplifying from to 9 to 22 DNA fragments (average = 13.8 ± 4). The profiles were examined for fragments that were unique and conserved among the individuals of a given geographic location, and could be used to generate a 'diagnostic profile' (Kambhampati *et al.*, 1992). Only a few fragments were unique to a given geographic sample: six proved exclusive to the three Marettimo individuals, one to the Madonie and zero to Ustica and Tufanio. Primers OPF-06 and OPF-02 followed by OPF-03 and OPF-07 proved able to amplify the higher number, with respect to the ten screened primers, of rare and polymorphic conserved DNA fragments in the set of specimens considered.

Figure 4 reports the UPGMA dendrogram of the association among the 12 specimens. Three distinct clusters are formed, and the Marettimo sample is separated at a relatively lower level of similarity from the other nine *C. sicula* (SM = 0.5). At a very close level (SM = 0.53) the dendrogram shows the separation between the northeastern Madonie sample and the northwestern

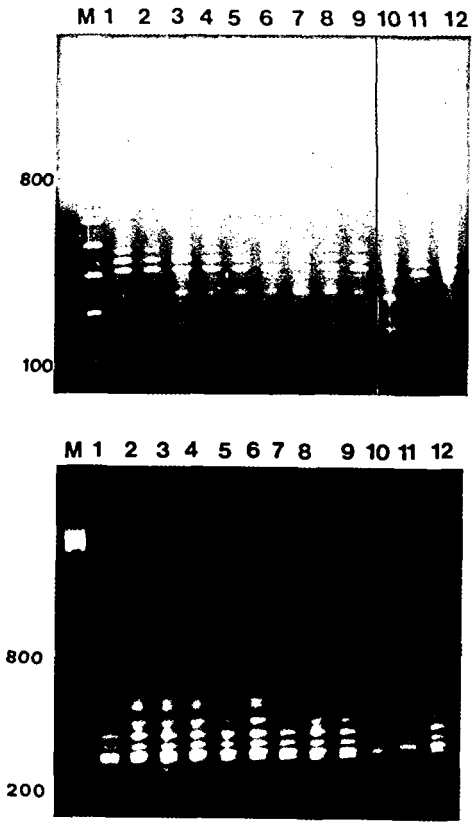

Fig. 3 - RAPD amplifications with primer OPF 2 (above), showing the exclusive bands for the Madonie in positions around 800 bp, and OPF-10 (below), showing an amplification example of a high number of monomorphic bands. Each lane consists of DNA from a single shrew. 1-3: *Crocidura sicula* from Marettimo (MAR); 4-6: *C. sicula* from Tufanio (TUF); 7-9: *C. sicula* from Ustica (UST); 10-12: *C. sicula* from Madonie (MAD); M, standard weight marker in base pairs.

Tufanio one, plus the Ustica shrews, which are strictly associated to the latter sample and behave as a distinct cluster inside the Tufanio one.

A similar UPGMA dendrogram (Fig. 5) was obtained when the Nei distance, based on the mean gene frequency of each sample of three specimens, was considered; the Marettimo sample was again the most distant from the other *C. sicula*, and those of Tufanio and Ustica were the most similar with respect to the northeastern Madonie one.

The UPGMA of the Mahalanobis phenetic distances (Fig. 6) resulted in a somewhat different clustering; the shrews from the two small islands are well separated from the two Sicilian populations, and this is consistent with the results of broader analyses carried out on the *C. sicula* morphometrics (Sarà, 1995). The correlation between the Nei and the Mahalanobis matrices ($r = 0.27$, and the derived Mantel test statistic, $Z = 0.75$, $P = NS$), showed a very poor fit between genetic and morphometric variation in the four geographic localities.

The Hj values (Table III) show the mean observed gene diversity within each sample, the Madonie giving the highest value, followed by Tufanio and Marettimo and

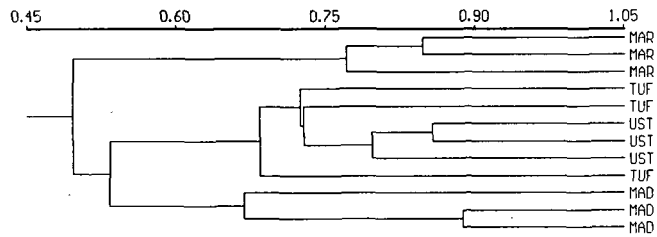

Fig. 4 - UPGMA dendrogram of simple matching coefficient (similarity) among the 12 shrews. The three *Crocidura sicula* from Marettimo have the lowest similarity with respect to the cluster formed by the Ustica and the two Sicilian samples (Tufanio, northwestern and Madonie, northeastern). Abbreviations as in Figure 3. The cophenetic correlation coefficient ($r = 0.86$) indicates a good fit and the statistically significant Student t-test ($t = 6.93$; $P < 0.001$) rejects the null hypothesis that the observed UPGMA clustering is random.

finally by Ustica. Even allowing for the possible bias due to the small sample size, the two small islands show a reduced heterozygosity.

Table IV shows the body biometrics variation, which in Crocidurinae is not affected by sexual dimorphism, of the *C. sicula* living in the different islands of their range. The biometrics of the Ustica shrew indicates the presence of a slightly larger population, not significantly different, except for total body length, from the Sicilian shrews (cf. min-max values in Table IV). The Marettimo shrews, on the contrary, show a significant reduction of body size. The pair-wise comparison between Marettimo and Ustica populations is also statistically different, except for hindfoot length.

There is, however, an important phenotipic feature, other than morphometrics, which distinguishes the Ustica shrews (Fig. 1). These animals have a darker and brownish colouration, whereas the Marettimo (and Gozo, see Vogel *et al.*, 1990) shrews have the typical grey-whitish colour pattern described in Sicily (Vogel, 1988).

DISCUSSION

Black IV (1993), reviewing the employment of RAPD-PCR in systematics of insects, pointed out several problems that might arise when applying this technique to molecular taxonomy. In particular, he stressed two major drawbacks: poor reproducibility due to the

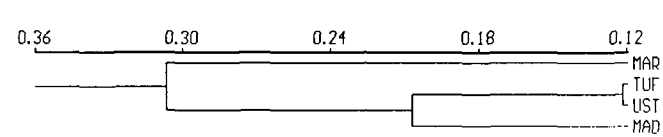

Fig. 5 - UPGMA dendrogram of Nei distances among the four populations. The Ustica shrews are genetically very close to the northwestern Sicilian sample (Tufanio), while the Madonie (northeastern Sicily) shrews join with the former samples at a farther distance. The three Marettimo shrews are well separated from the others. Abbreviations as in Figure 3. The cophenetic correlation coefficient $r = 0.90$ ($t = 1.80$; $P < 0.05$).

TABLE II - *Number of fragments amplified by each random primer. Only the rare polymorphic bands were analysed.*

Primer	Sequence 5' to 3'	Total bands	Monomorphic bands	Common polymorphics $f \geq 0.75$	Rare polymorphics $f < 0.75$	Bands exclusive to Tufanio	Bands exclusive to Ustica	Bands exclusive to Marettimo	Bands exclusive to Madonie
OPF-01	ACGGATCCTG	11	0	2	9	0	0	0	0
OPF-02	GAGGATCCCT	19	4	2	13	0	0	0	1
OPF-03	CCTGATCACC	14	0	2	12	0	0	0	0
OPF-04	GGTGATCAGG	9	1	0	8	0	0	1	0
OPF-05	CCGAATTCCC	14	1	6	7	0	0	0	0
OPF-06	GGGAATTCGG	22	0	2	20	0	0	5	0
OPF-07	CCGATATCCC	13	0	2	11	0	0	0	0
OPF-08	GGGATATCGG	12	1	4	7	0	0	0	0
OPF-09	CCAAGCTTCC	14	1	4	9	0	0	0	0
OPF-10	GGAAGCTTGG	10	6	1	3	0	0	0	0
TOTAL		138	14	25	99	0	0	6	1

possible presence of artifacts and the fact that two co-migrating bands might not be constituted by the same 'allele', especially when analysing taxonomic units that are far apart. In order to overcome the first of these problems, we employed the Stoffel fragment of Taq polymerase, which is reported (Lawyer *et al.*, 1993) to optimize the reproducibility of RAPD analysis. Our experiments were repeated several times and we observed overall a very good level of reproducibility. Patterns that were not reproducible were excluded.

The second problem, the fact that two co-migrating bands might have the same length but not the same sequence, remains of course unsolved. This problem also exists, however, when comparing protein patterns. The ultimate solution could come from sequencing, but even in this case the possibility of insertion of a wrong base by the polymerase does indeed exist. We believe that this problem should not seriously affect our results, because working at intraspecific level the probability of two different co-migrating DNA fragments is minimal (Rieseberg, 1996).

This insight into the molecular variability of the endemic *C. sicula* can be said to give a first account of the microevolutive processes occurring in the insular range of the species, which can be summarized as follows:

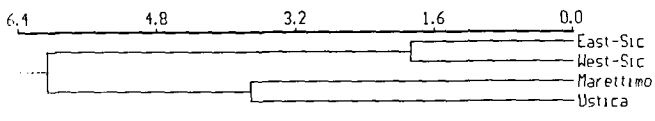

Fig. 6 - UPGMA dendrogram of the Mahalanobis matrix (phenetic distances) among two geographic samples from Sicily and the two surroundings island. The Mahalanobis matrix was obtained by the canonical variate analysis of 14 metric variables taken on 74 skulls. The Ustica and Marettimo shrews proved to be phenetically well separated from the Sicilian populations. The cophenetic coefficient $r = 0.97$; ($t = 1.57$; $P = 0.05$).

Phenotypic and genic variability

The phenotypic and morphological features, so far described in the *C. sicula* populations, are in one case (Marettimo) and not in another (Ustica) consistent with the RAPD-marker differences here reported. Also by geographic variation analysis (Sarà, 1995), Marettimo and Ustica are well separated in the multivariate morphometrics space (i.e., each island has its own distinctness), even though they follow the same general trend of size reduction and shape change of the skull, i.e. the two small islands are pooled together apart from Sicily.

The lack of correlation between the Nei (Fig. 5) and the Mahalanobis (Fig. 6) matrix proved that each population behaves independently in its process of morphological and molecular evolution. Schnell & Selander (1981) pointed out that genic and morphological evolution seems to be independent in mammals, and the nature and magnitude at these different evolutive processes may thus, or may not be totally consistent (Patton, 1985).

TABLE III - *Mean observed gene diversity (Hj) within populations for the four geographic samples of* Crocidura sicula.

	n	Hj*
Marettimo	3	0.119
Tufanio	3	0.159
Ustica	3	0.099
Madonie	3	0.144
Sicily	6	0.259

*, Lynch & Milligan, 1994.

TABLE IV - *Body biometrics (*mean ± *standard deviation, min-max values) of* Crocidura sicula *in mainland Sicily and surrounding islands. The Student* t *tests express the significancy between samples for a given variable.*

	Weight (g)	Total body length (mm)	Tail length (mm)	Hindfoot length (mm)
Sicily (unpubl. plus Sarà *et al.*, 1990)	6.9 ± 1.16 (5.0-9.5); n = 47	69.8 ± 4.7 (63.0-79.0); n = 24	36.6 ± 3.74 (32.0-45.0); n = 23	12.3 ± 0.37 (11.5-13.0); n = 24
CV	16.81%	6.73%	10.1%	3.01%
Egadi (unpubl. plus Krapp, 1969)	6.1 ± 1.24 (4.5-8.5); n = 11	65.3 ± 3.6 (61.5-71.5); n = 9	33.3 ± 1.20 (32.0-35.5); n = 9	11.4 ± 0.34 (11.0-12.0); n = 8
CV	20.33%	5.51%	3.60%	3.25%
Ustica (unpubl.)	7.5 ± 0.95 (7.0-9.0); n = 5	72.0 ± 6.1 (68.0-79.0); n = 3	37.8 ± 1.79 (36.0-40.0); n = 5	12.2 ± 0.30 (11.9-12.5); n = 3
CV	12.67%	8.5%	4.7%	2.5%
Gozo (Schembri & Schembri, 1976)	ND	50.3 n = 1	27.5 n = 1	11.6 n = 1
t Student Sicily/Marettimo	2.25 P < 0.05	7.71 P < 0.001	6.24 P < 0.001	2.75 P = 0.01
t Student Sicily/Ustica	1.21 P = NS	2.33 P < 0.05	1.75 P = NS	0.20 P = NS
t Student Ustica/Marettimo	2.35 P < 0.05	6.42 P < 0.001	6.87 P < 0.001	1.41 P = NS

NS, not significant; CV, coefficient of variation; ND, not determined.

DNA fingerprinting data, so far, do not show a large genetic divergence between the Ustica and the mainland Sicilian shrews, whereas the fur colour (Fig. 1) and skull morphometrics (Fig. 6) of these animals are greatly different.

Melanism has not yet been described in the Crocidurinae (Churchfield, 1990; Vogel, *in litteris*), but different colourations, such as erythrism and melanism, are cited as a common feature of *Lacerta (Podarcis)* or *Gongilus* lizards in Mediterranean volcanic islands (La Greca & Sacchi, 1957; Sacchi, 1961). The melanism insorgence has been proposed (Sacchi, 1961; Margaleff, 1961) as a consequence of the erosion of genetic variability, caused by directional (mimicry or adaptation to insularity) or neutral (genetic drift and founder effect) selection. Gorman *et al.* (1975), by allozyme studies on some small-island lizard populations, demonstrated that the observed low allelic diversity is a function of the island's ecological distinctness, and that the loss of genetic variation is a consequence of the prevailing directional selection.

For the island of Marettimo, DNA fingerprinting data show a larger genetic distance with respect to the mainland Sicilian shrews, and a parallel variation in skull morphometrics and body biometrics. According to the above hypotheses, this island could have been colonized by 'normal-coloured' small Sicilian founders or by animals which were later selected towards smaller body size and different skull morphometrics. It is well known that insular vertebrate populations are affected by giantism and dwarfism, and Case (1978) gave some indications about their general body size trends. In *C. sicula*, the Ustica population shows a non significant trend towards an increase of body size rather than a significant decrease, such as occurs in the island of Marettimo. Other shrew populations in Mediterranean islands generally became bigger than their continental counterparts, as cited for *C. suaveolens* from Corsica (Poitevin *et al.*, 1987) or for *C. russula* from some Mediterranean islands (Sarà & Vogel, 1996).

Dynamics of colonization

The dynamics of the colonization can be tentatively reconstructed by the paleogeography of these islands. The Egadi is a calcareous archipelago, which formed together with Sicily and the Maltese archipelago, a single large island during the Pleistocene. This event occurred at least twice (La Greca, 1961; Thake, 1985), first during Middle Pleistocene, and later during Upper Pleistocene. Later, at the end of the Würmian marine regression, Marettimo (the farthest away of the Egadi, 35 km off the Sicilian coasts) and the Maltese were the first islands to separate (Fig. 2). Ustica is a volcanic island, which is isolated from Sicily by a deep (max depth 2000 m b.s.l.) and wide (67 km) sea channel. It emerged at least

1 000 000 years B.P. and has always been separate from Sicily. Its terrestrial fauna is characterized by recent immigrant taxa (Massa & Di Palma, 1988), whose occurrence is best explained by rafting or anthropogenic introduction during the Neolithic. The first record of human colonization on Ustica dates back no more than 1500-1300 B.C.

According to the above paleogeographic history, one can hypothesize that the Marettimo shrews could have been separated from their conspecifics at least since 30 000-10 000 years B.P., whereas the Ustica shrews might have come on the island in historical times, at least 3 500-3 000 years ago, by accidental anthropogenic introduction.

Even though chronological parameters cannot be predicted by RAPDs, whose rate of evolution cannot be established, nevertheless we can observe that the hypothesis of long isolation of the Marettimo shrews from their Sicilian counterparts is in qualitative agreement with the RAPD profile (Figs 4 and 5) in that some primers evidenced six fragments of conserved DNA unique for this population (Table II). Within this context, a very reduced or even absent gene flow, i.e. other colonizations, is likely to have occurred since the earlier separation of Marettimo. As regards to the Ustica shrews, the lack of exclusive DNA fragments and their closer genetic distance to the Tufanio sample is also in agreement with the hypothesis of a more recent arrival/isolation of these shrews. Alternatively for Ustica, it is also possible, but it seems unlikely when considering the strikingly different phenotype, that, because of several accidental arrivals, gene flow from Sicily has not been interrupted since the first introduction of the species.

CONCLUSIONS

Electrophoretical data based on the gene-enzyme system (e.g. Ayala *et al.*, 1972; Sarich, 1977) have been used to make correlations among the infraspecific and specific taxonomic categories and the genetic variability and distance of populations, by the standard Nei (1972, 1978) measurements, as well as to estimate the time of evolutionary divergence. In the case of shrews (see Maddalena, 1990), according to the standardizations by allozyme studies, the threshold to assign a population to different species would be a D close to 0.1, whereas lower values would indicate conspecificity, as well as infraspecific variation (D = 0.05). Nei's genetic distance coefficients applied to the resulting set of RAPD-PCR data have shown, so far, a higher magnitude than those from electrophoretical data. This, coupled with the small sample size used, does not yet permit any formalization in taxonomic ranks. However, it is interesting to point out that: i) the dendrograms obtained for *C. sicula* are consistent with the biogeography of these shrews, thus confirming the validity of this technical approach; ii) the melanic shrews from Ustica proved to be strictly related, on a genetic ground, to those of mainland Sicily.

In conclusion, the shrews in Marettimo and Ustica can be considered to be affected by an independent divergence, both on phenotypic and genotypic grounds, and represent two separately evolving isolated populations. The particular and pronounced phenotypic plasticity of the *C. sicula* living in Ustica, i.e. the presence of melanism, is probably related to a strong selection and/or ecological adaptation due to the volcanic environment.

REFERENCES

Arnold M. L., Buckner C. M., Robinson J. J., 1991 - Pollen-mediated introgression and hybrid speciation in Louisiana irises. Proc. Natl. Acad. Sci. USA, *88*: 1398-1402.

Ayala F. J., Powel J. R., Tracey M. L., Mourão C. A., Pérez-Sala S., 1972 - Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics, *70*: 113-139.

Black IV W. C., 1993 - PCR with Arbitrary Primers: Approach with Care. Insect mol. Biol., *2*: 1-6.

Case T.J ., 1978 - A general explanation for insular body trends in terrestrial vertebrates. Ecology, *59*: 1-18.

Catalan J., Poitevin F., 1981 - Les Crocidures du midi de la France: leurs charactéristiques génétiques et morphologiques; la place des populations corses. C. R. Acad. Sci. Paris, *292*: 1017-1020.

Catzeflis F., 1983 - Relations génétiques entre trois espèces du genre *Crocidura* (Soricidae, Mammalia) en Europe. Mammalia, *47*: 229-236.

Churchfield S., 1990 - The natural history of shrews. Croom Helm, London, 178 pp.

Gorman G. C., Soulè M., Yang S. Y., Nevo E., 1975 - Evolutionary genetics of insular Adriatic lizards. Evolution, *29*: 52-71.

Hadrys H., Balick M., Schierwater B., 1992 - Application of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol., *1*: 55-63.

Hadrys H., Schierwater B., Della Porta S. L., De Salle R., Buss L. W., 1993 - Determination of paternity in dragonflies by Random Amplified Polymorphic DNA fingerprinting. Mol. Ecol., *2*: 79-87.

Herrmann B. G., Frischauf A. M., 1987 - Isolation of genomic DNA. Methods Enzymol., *152*: 180-183.

Hutterer R., 1981 - Der Status von *Crocidura ariadne* Pieper, 1979 (Mammalia: Soricidae) Bonn. zool. Beitr., *32*: 3-12.

Kambhampati S., Black IV W. C., Rai K. S., 1992 - Random amplified polymorphic DNA of mosquito species and populations (Diptera: Culicidae): techniques, statistical analysis and applications. J. med. Entomol., *29*: 939-945.

Krapp F., 1969 - Terristrische Kleinsäugetiere von den ägadischen Inseln (Mammalia: Insectivora, Rodentia) (Provinz Trapani, Sizilien). Mem. Mus. civ. St. nat. Verona, *17*: 331-347.

La Greca M., 1961 - Considerazioni sull'origine e la costituzione della Fauna di Sicilia. Arch. bot. biogeogr. ital., *6*: 2-23.

La Greca M., Sacchi C. F., 1957 - Problemi del popolamento animale nelle piccole isole mediterranee. Ann. Ist. Mus. Zool. Univ. Napoli, *9*: 1-190.

Lawyer F. C., Stoffel S., Saiki R. K., Chang S. Y., Landre P., Abramson R. D., Gelfand D. H., 1993 - High-level expression, purification, and enzymatic characterization of full-length *Thermus aquaticus* DNA polymerase and a truncated form deficient in 5' to 3' exonuclease activity. PCR Methods and Applications, *2*: 275-287.

Lynch M., Milligan B. G., 1994 - Analysis of population genetic structure with RAPD markers. Mol. Ecol., *3*: 91-99.

Maddalena T., 1990 - Systematics and biogeography of Afrotropical and Palearctic shrews of the genus *Crocidura* (Insectivora: Soricidae) an electrophoretic approach. *In*: G. Peter & R. Hutterer (eds), Vertebrates in the tropics. Museum A. Koenig, Bonn, pp. 297-308.

Margaleff R., 1961 - Modalités de l'évolution en rapport avec la simplification des biocénos insulaires. *In:* Le peuplement des Iles Méditerranéennes et le problème de l'insularité. Ed. du CNRS, Paris, pp. 313-318.

Massa B., Di Palma M. G., 1988 - Rettili, anfibi e uccelli terrestri delle isole circum-siciliane. Bull. Ecol., *19:* 225-234.

Miller G. S., 1901 - Five new shrews from Europe. Proc. biol. Soc. Wash., *14:* 41-45.

Nei M., 1972 - Genetic distance between populations. Am. Nat., *106:* 283-292.

Nei M., 1978 - Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, *89:* 583-590.

Patton G. L., 1985. Population structure and genetic of speciation in pocket gophers, genus *Thomomys*. Acta zool. fenn., *170:* 109-114.

Poitevin F., Catalan J., Fons R., Croset H., 1987 - Biologie évolutive des populations ouest-européennes de Crocidures (Mammalia, Insectivora). II. - Ecologie comparée de *Crocidura russula* Hermann, 1780 et de *Crocidura suaveolens* Pallas 19811 dans le midi de la France et de la Corse: rôle probable de la competition dans le partage des milieux. Rev. Ecol. (Terre Vie), *42:* 39-58.

Rieseberg L. H., 1996 - Homology among RAPD fragments in interspecific comparisons. Mol. Ecol., *5:* 99-105.

Rohlf F. J., 1993 - NTSYS-pc - Numerical taxonomy and multivariate analysis system, Version 1.80. Exeter Software, New York, 239 pp.

Sacchi C. F., 1961 - Considérations sur le phénomènes micro-évolutifs animaux des petites iles méditerranéennes. *In:* Le peuplement des Iles Méditerranéennes et le probleme de l'insularité. Ed. du CNRS, Paris, pp. 321-335.

Sarà M., Lo Valvo M., Zanca L., 1990 - Insular variation in Central Mediterranean *Crocidura* Wagler, 1832. Boll. Zool., *57:* 283-293.

Sarà M., 1995 - The Sicilian (*Crocidura sicula*) and the Canary (*C. canariensis*) shrew (Mammalia, Soricidae); peripheral isolate formation and geographic variation. Boll. Zool., *62:* 173-182.

Sarà M., Vitturi R., 1996 - *Crocidura* (Mammalia, Soricidae) populations from the Sicilian-Maltese insular area. Hystrix, *8:* (in press).

Sarà M., Vogel P., 1996 - Geographic variation of the greater white-toothed shrew *Crocidura russula* Hermann, 1780 (Mammalia, Soricidae). Zool. J. linn. Soc., *116:* 377-392.

Sarich V. M., 1977 - Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. Nature (Lond.), *265:* 24-28.

Schembri P. J., Schembri S. P., 1979 - On the occurrence of *Crocidura suaveolens* Pallas (Mammalia, Insectivora) in the Maltese Islands with notes on other Maltese shrews. Cent. Mediterr. Nat., *1*: 18-21.

Schnell G. D., Selander R. K., 1981 - Environmental and morphological correlates of genetic variation in mammals. *In:* M. H. Smith & J. Joule (eds), Mammalian population genetics. University Georgia Press, Athens, pp. 60-99.

Taberlet P., Fumagalli L., 1996 - Owl pellets as a source of DNA for genetic studies of small mammals. Mol. Ecol., *5:* 301-306.

Thake M. A., 1985 - The biogeography of the Maltese islands, illustrated by the Clausiliidae. J. Biogeogr., *12:* 269-287.

Toschi A., 1959 - Insectivora. *In:* A. Toschi & B. Lanza (eds), Fauna d'Italia, vol IV: Mammalia. Calderini, Bologna, pp. 1-186.

Vesmanis I., 1976 - Beitrag zur Kenntnis der Crociduren-Fauna Siziliens (Mammalia: Insectivora). Z. Säugetierk., *41:* 257-273.

Vogel P., 1988 - Taxonomical and biogeographical problems in Mediterranean shrews of the genus *Crocidura* (Mammalia, Insectivora) with reference to a new karyotype from Sicily (Italy). Bull. Soc. Vaud. Sci. nat., *79:* 37-48.

Vogel P., Hutterer R., Sarà M., 1989 - The correct name, species diagnosis and distribution of the Sicilian shrew. Bonn. zool. Beitr., *40:* 243-248.

Vogel P., Maddalena T., Catzeflis F., 1986 - A contribution to the taxonomy and ecology of shrews (*C. zimmermanni* and *C. suaveolens*) from Crete and Turkey. Acta theriol., *31:* 537-545.

Vogel P., Schembri P. J., Borg M., Sultana J., 1990 - The shrew (*Crocidura* sp.) of Gozo, a probable survivor of the Pleistocene fauna of Mediterranean islands. Z. Säugetierk., *55:* 357-359.

Wettstein O., 1925 - Beiträge zur Säugetierkunde Europas I. Arch. Naturgesch., *91:* 139-163.

Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A., Tingey S. V., 1990 - DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Ac. Res., *18:* 6531-6535.