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CROCIDURA POPULATIONS (MAMMALIA, SORICIDAE) FROM THE SICILIAN-MALTESE INSULAR AREA

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ABSTRACT – Recent studies have shown the presence of a new endemic species of Soricidae, *Crocidura sicula,* in the Mediterranean area. Its karyotype consists of 36 chromosomes (NF = 56, NFa = 52) as previously recorded in specimens from Sicily and Gozo (Malta). Karyological analysis carried out in the present study on specimens from Marettimo (Egadi) and Ustica and on two additional Sicilian localities proved the presence of the same C. *sicula* karyotype. The multivariate analysis of skull and mandible of these populations revealed a pattern of geographic variation which is mainly related to a size decrease (76% of the total variability accounted for) in shrews living in the small islands around Sicily. The body biometrics of these insular shrews follows this trend, with some exceptions such as in Ustica, where shrews are relatively bigger. The shrews from Ustica show also melanic fur, which is darker than the grey-witheish colour found in conspecific populations. Such a phenotype could be the result of adaptation to the volcanic environment on this island. This latter population is, of all the small islands, the most divergent and differentiated from the mother-island; if considering its skull morphometrics (i.e. the residual 24% of total variability) and its body biometrics.

***Key** words: Crocidura sicula,* Karyotype, Morphometrics, Ustica, Marettimo.

INTRODUCTION

The taxonomy of the species of *Crociduru* occurring in Sicily has been a matter of discussion for a long time. Both the number of species (up to three, according to some Authors) recorded on this island and their taxonomic attribution have been questioned. The status of the Maltese (Gozo) population has also provided controversy (Schembri and Schembri, 1979), while the shrews living in the Egadi archipelago (Levanzo, Marettimo and Favignana) were attributed to *Crociduru suaveolens* (Krapp, 1969; Vesmanis, 1976).

Recently, karyological studies (Vogel 1988; Vogel et al., 1990) showed the occurrence of a new karyotype shared by the Sicilian and Maltese (Gozo) shrews. This allowed the description of a new endemic species, *Crociduru sicula* Miller, 1901 (Vogel et al., 1989).

The contemporary study on geographic variation by multivariate morphometrics (Sarh et al., 1990; Sarh, 1995) confirmed the existance of a single species in Sicily and in some surroundings small islands (Gozo, Ustica, Levanzo, Marettimo and Favignana), and evidenced the morphometric characteristics and the distinction of the taxon *sicula* with respect to the other Palearctic species (C. *russula,* C. *leucodon,*

C. *suaveolens*, *C. whitakeri*, C. *canariensis*, *C. sicula)* and to a fossil form from the Middle Pleistocene of Sicily (C. *esuae).*

To investigate in detail the geographic variation of the *Crocidura sicula* of the Sicilian-Maltese insular area, we report here the morphometrics of their skull and mandible, and we give details of their external phenotypes and body biometrics. We report also the karyotypes of two new insular populations (Marettimo and Ustica) and of two Sicilian locations.

MATERIAL AND METHODS

To analyze the biometric variation and the morphometric relationships among the C. *sicula* insular populations, part of the theriological collection of the Zoological Museum of the University of Palermo (MZPA, Palermo, Italy), which includes more than 600 *Crocidura* specimens from Europe, was used. The mandibles and skulls were measured with a stereomicroscope fitted with micrometric lenses. A detailed description of the metric variables used (four for the mandible and fourteen for the skull) and of their multivariate analyses is reported in Sarà et al. (1990) and Sarà (1995). The linear measurements for the mandible were collected from 262 specimens from six islands, which were considered as Operational Taxonomy Unities (OTUs); and for the skull, on 124 specimens coming from five OTUs (Tab. 1).

Table 1 Origin and composition of samples used in multivariate analyses. N= total number of specimens. PCA = Principal Component Analysis.

TAXON	OTU	PCA MANDIBLE, 4 METRIC VARIABLES	PCA SKULL, 14 METRIC VARIABLES
C. *sicula*	FAVIGNANA	**16**	**6**
C. *sicula*	LEVANZO	***6***	*0*
C. sicula	GOZO	**17**	**10**
C. sicula	MARETTIMO	**16**	**20**
C. sicula	USTICA	**12**	**13**
C. sicula	SICILY	**195**	**75**
N		**262**	**124**

The separate analysis of the mandible and the skull allowed the introduction of the Levanzo sample, which is represented by mandibles only. To analyze the Sicilian intrapopulational variation, the skull sample was divided in seven geographic localities: 1 - North-East (Nebrodi mountains and Etna), 2 - South-East (Gela plain), 3 - Central (Sperlinga woods), 4 - Mid-West (Sicani mountains and Ficuzza woods), 5 - South-West (Belice valley), 6 - North-West (Palermo mountains), 7 - West (Trapani Province).

Twenty-nine living specimens were trapped during spring-summer 1990-1992, using special traps (Trip-trap model, HYWARE, North West Plastics, Ltd), at Marettimo, Ustica, and in three Sicilian mainland localities (Campofelice di Roccella - Pa; Gratteri Pa; Alcamo

- Tp). Seven of them were killed for karyological analysis. Chromosome preparations were obtained by the air-drying technique on solid tissue (Vitturi, 1992). Slides from specimens of the four localities were stained using 5% Giemsa solution (pH 6.8) for the analysis of the standard karyotype. Chromosome preparations of specimens from Marettimo and Campofelice di Roccella were also treated using the C-banding technique described by Sumner (1972).

To estimate the relative frequency (or activity density) of shrews in each sampling locality, the DAT index (Pucek, 1969) was used, corresponding to [no. trapped individuals/(n trapping nights x n used traps)] x 100. The PFI indicates the % frequency of shrews remains in the Barn Owl (*Tyto alba*) pellets in the same localities (Tab. 2).

All the available data on the body biometrics of C. *sicula* were collected from literature (cfr. Krapp, 1969; Schembri and Schembri, 1976; Sarà et al., 1990), from trapping (living weight in gr.) and from the MZPA Mammal collection (total-body, tail and hindfoot lengths in mm. from alcohol specimens). To detect differences in the body biometrics, the Student's t test was performed to compare insular populations.

Table 2 Specimens recorded in Barn Owl pellets or trapped in the 4 sites considered for chromosome analysis. PFI = Frequency in pellets; DAT = trapping frequency; na = not available.

	MARETTIMO	USTICA	CAMPOFELICE	ALCAMO
PELLETS	27	52	85	na
N° PREYS	169	208	229	na
Crocidura sicula	46	42	13	na
PFI%	27.22	20.19	5.68	na
TRAP-NIGHTS	84	391	1335	67
Crocidura sicula	4	3	19	3
DAT%	4.76	0.77	1.42	4.48

RESULTS

KARYOTYPE

The conventional karyotype was the same in all four geographic localities studied. It include seventeen homomorphic pairs, five of which were metacentric, five submetacentric or subtelocentric and seven acrocentric; plus a heterochromosome pair (XX in females and XY in males). In this pair, the X is a large sized metacentric while the Y is a medium-large sized subtelocentric chromosome (Fig. 1). The karyotype which was found in Sicily, Marettimo and Ustica resembles those reported (Vogel, 1988) in a single specimen from Isnello (near Campofelice di Roccella) and in specimens from Gozo (Vogel et al., 1990). They displayed: 2n = 36, NF = 56, NFa = 52.

Slight morphological differences among the karyotypes of these localities, involving one or two chromosome pairs, might be due to the fact that the arm ratio

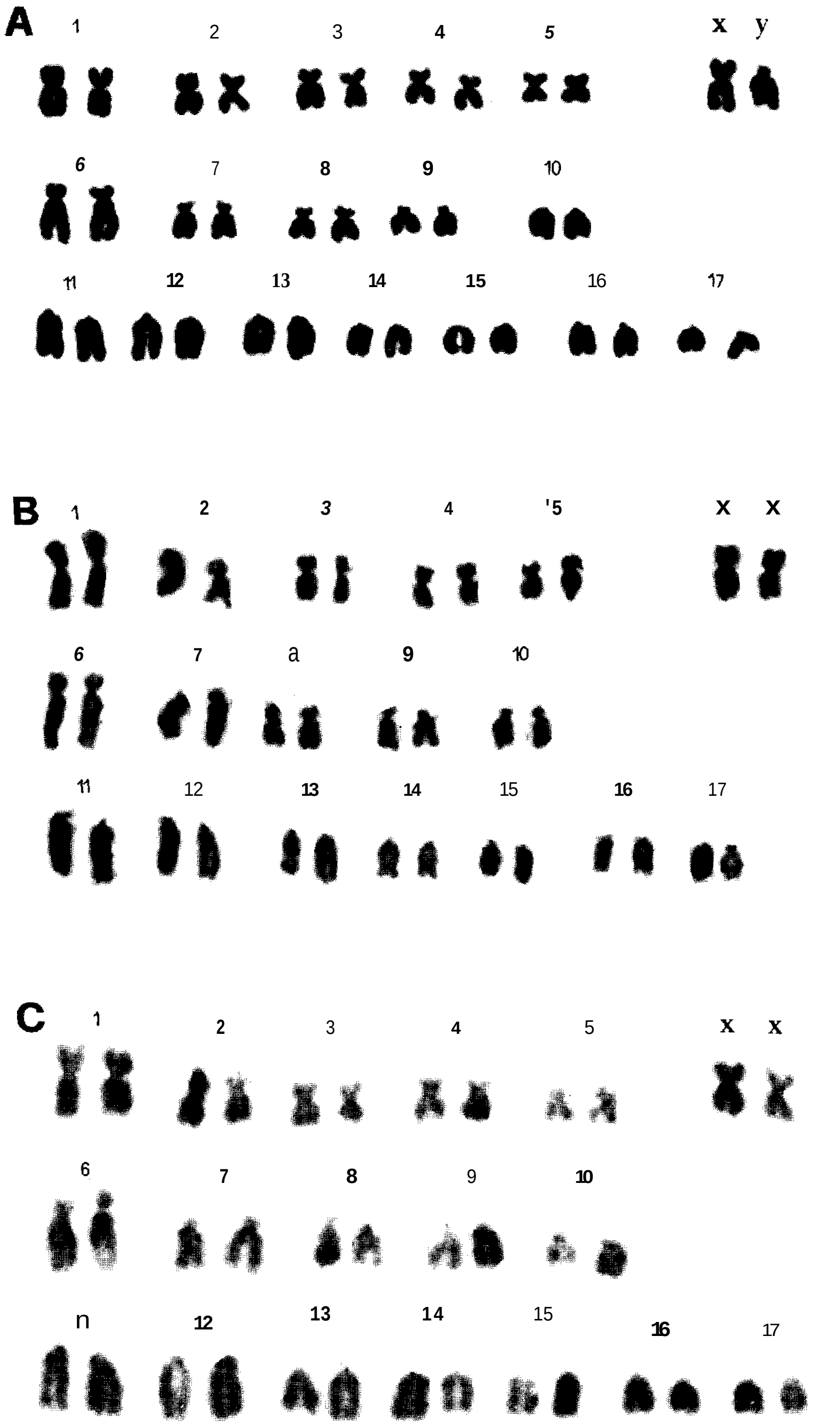

Figure 1 Giemsa stained karyotype of *C. sicula*: **A** = male from Marettimo (Egadi); B = female from Campofelice di Roccella (Sicily); C = female from Ustica.

of some chromosomes are borderline between submetacentric and subtelocentric.

Application of the C-banding technique (Fig. 2) carried out on two specimens (1 from Campofelice di Roccella and 1 from Marettimo) showed consistent C-heterochromatin banding, located terminally in the second pair. This might correspond to the nucleolus organizer region (NOR). **All** the other chromosome pairs are characterized by minute centromeric C-positive bands.

Further analyses by other banding techniques such as, silver nitrate and fluorocromatine staining, on more individuals are required to check if there are any karyological differences among these micro-insular and the Sicilian populations.

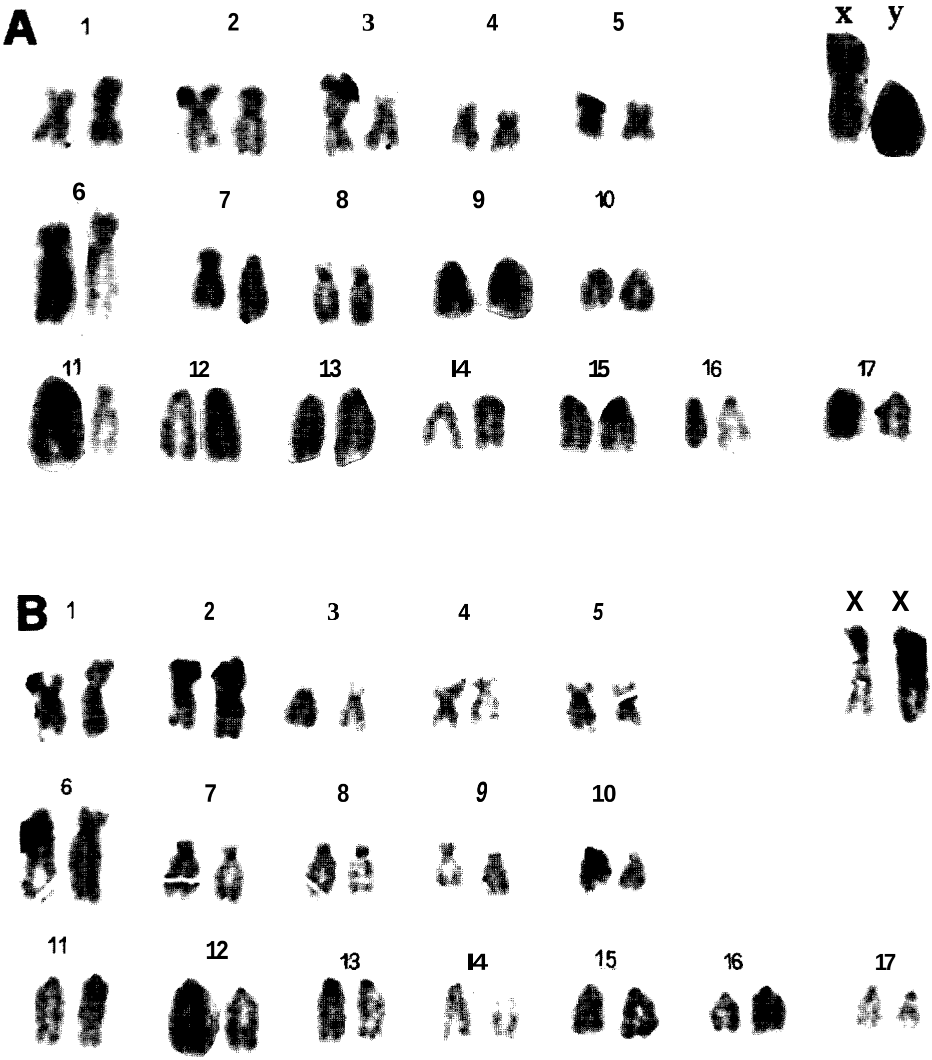

Figure 2 C-banded karyotype of *C. sicula*: **A** = male from Marettimo (Egadi); B = female from Campofelice di Roccella (Sicily)

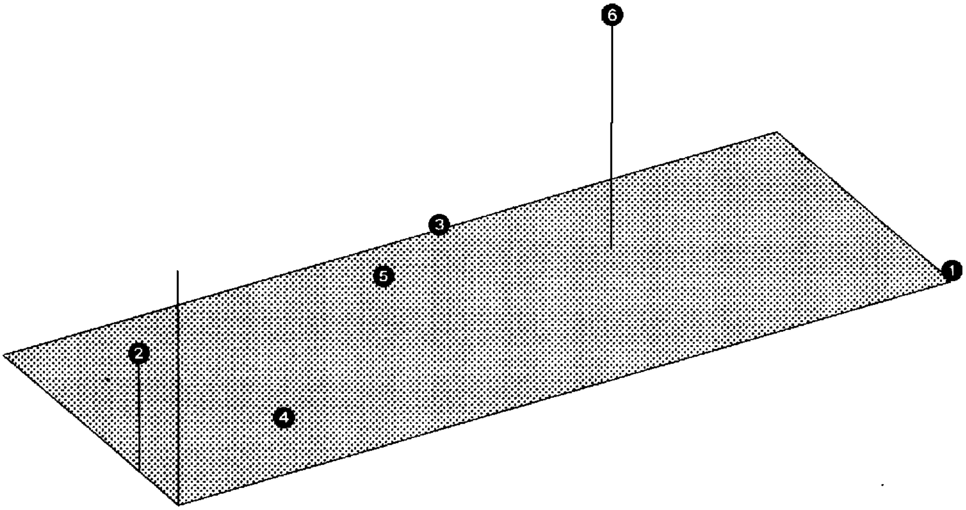

Figure 3 - Scatter plot of the centered-PCA on the shrew mandibles (C. *sicula*). The plot shows the first three components (F1 = 75.95% of total variance, F2 = 13.95%, and F3 = 9.42%) and suggests an ordination according to a "size-factor" along the F1 (above) and to shape along the F2 and F3 (below). The shrews from Sicily have the largest mandible and those from Favignana the smallest. The mandible shape of the Ustica shrews **is** the most different, whereas that of the Levanzo population is closer to Sicily. 1 = Sicily; 2 = Favignana; 3 = Marettimo; 4 = Levanzo; 5 = Gozo; 6 = Ustica.

The present result however, shows stability in the number and morphology of the standard karyotype in all the C. *sicula* specimens from Gozo, Ustica, Marettimo and Sicily.

MORPHOMETRICS:

The scatter plot of the **PCA** computed on the mandibles (Fig. 3) shows a different ordination between the Sicilian and the small islands samples; both for the known 36-chromosomes karyotype populations (Marettimo, Gozo, Ustica) and for the unknown karyotype (Levanzo, Favignana). The ordination along the first principal component (F1) accounts for 76% of the total variance and is, according to Jolicoeur and Mosimann (1960), a 'size-component'. The populations of C. *siculu* are thus ordinated according to their decreasing mandible size (from right to left). The Favignana and Levanzo shrews have the smallest mandible, and the Ustica shrews have, among the small insular population, the largest.

The contribution of the four metric variables to the F1-F3 axes formation is reported in Tab. 3. All the characters contribute highly (from 55% to 96.5%) to the 'size-component' formation.

Table 3 Variance percentage of variables (above mandible, below skull) accounted for by each component (F1-F3). (+) = positively correlated to a given component; (-) negatively correlated. L =length; W =width.

MANDIBLE	F1	F2	F3
Total L excluding incisor	96.5 (+)	1.6 (-)	0.05 (+)
L of molar row	75.3 (+)	2.6 (-)	21.8 (-)
width of articular condylum	55.1 (+)	44.7 (+)	0.2 (+)
height at the coronoid process	76.9 (+)	7.0 (-)	15.6 (+)
SKULL			
zygomatic W	79.3 (+)	12.1 (+)	3.0 (-)
palate **L**	96.3 (+)	1.8 (+)	0.8 (-)
upper tooth-row **L**	68.3 (+)	18.2 (+)	3.8 (+)
incisor **L**	88.5 (+)	1.9 (-)	0.02 (+)
L of the II° unicuspid	58.0 (+)	0.02 (-)	26.8 (+)
L of the III° unicuspid	84.0 (+)	2.7 (+)	0.3 (-)
external buccal **L** of the IV° premolar	87.0 (+)	3.3 (-)	3.8 (+)
internal palatal L of the IV" premolar	16.8 (+)	80.0 (+)	0.3 (+)
max W of the **I**" molar	77.2 (+)	0.2 (-)	12.1(-)
max W of the II° molar	71.4 (+)	16.3 (-)	4.3 (-)
max W of the III° molar	80.2 (+)	15.3 (-)	1.1 (+)
median L of the I°molar	90.0 (+)	0.1 (-)	0.2 (-)
median L of the II°molar	89.8 (+)	0.04 (-)	8.4 (-)
median L of the III° molar	72.7 (+)	7.1 (-)	6.9 (+)

The OTUs ordination according to the 'shape-components', i.e. the F2-F3 axes, accounts for only 24% of total variation and evidences the morphometric divergence of the Ustica sample. The mandible variables which have the higher loading on these axes are the articular condylum width and the molar row length.

The PCA scatter plot for the skull (Fig. 4) shows the same ordination among the seven Sicilian geographic locations and the four small islands. Also the ordination along F1 account for the 76% of total variance and is a 'size-component'. The C. *sicula* populations from Sicily have the largest skull and the Favignana shrews have, once again, the smallest. The other OTUs show an intermediate skull size. The seven locations from Sicily are clustered together and do not show any evidence of trends in geographic variation within the island, i.e. latitudinal or longitudinal clines, etc.

The contribution of the fourteen characters to the F1-F3 axes **is** reported in Tab. 3; all of them, apart from the internal palatal length, account for 58% to 96.3% of this size component.

The OTUs ordination according to the 'shape-components' shows, also in the case of skull, a morphometric divergence **of** the Ustica population; and to a lesser extent of the Gozo and Marettimo population. The variables which have the highest

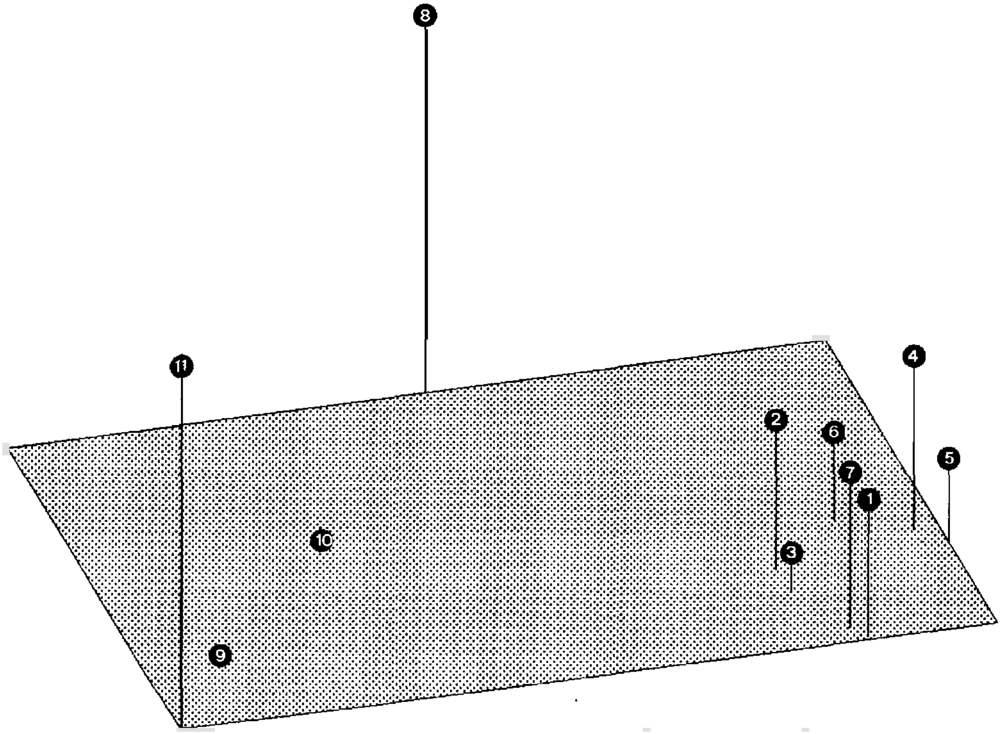

Figure 4 - Scatter plot of the centered-PCA on the shrew **skull** (*C. sicula*). The plot shows the first three components (F1 = 75.70% of total variance, F2 = 11.30%, and F3 = 5.10%) and suggests an ordination according to a "size-factor" along the F1 (above) and to shape along the F2 and F3 (below). The populations (1-7) living in Sicily have a skull size larger than that of shrews living in small islands (8-11). The **skull** shape of the Ustica and Gozo shrews is divergent from the Sicilian morphotype, in whose variability the Favignana sample falls. I = North-East Sicily (Nebrodi mountains and Etna); 2 = South-East (Gela plain); 3 = Central (Sperlinga woods); 4 = Mid-West (Sicani mountains and Ficuzza woods); 5 = South-West (Belice valley); 6 = North-West (Palermo mountains); 7 = West (Trapani Province); 8 = Favignana; 9 = Marettimo; 10 = Gozo; 11 = Ustica.

loading on F2 are the internal palatal length of the IV premolar, the zygomatic width, the upper tooth-row length, the maximum width of the **11** and III molars; and on F3: the length of II unicuspid and the maximum width of the I molar.

EXTERNAL PHENOTYPE:

The body biometrics of the Egadi shrews reported in Krapp (1969), were already found to be significantly smaller (Student's t test) than the Sicilian shrews (Sarà et al., 1990). The single specimen from Gozo, reported in Schembri and Schembri (1976), does not allow any statistical test, but is even smaller than the Egadi specimens. It could be in fact a young animal.

The *C. sicula* body biometrics, up *to* date to 1995, is reported in Tab. 4; the specimens from Marettimo showed values similar to those reported in Krapp (1969) and were considered together in a single Egadi sample, always significantly different from the Sicilian sample.

Table 4 Body biometrics (mean ± standard deviation, min-max values) of the Sicilian shrew in mainland Sicily and surrounding islands. The Student t tests express the significance between populations for a given variable. ns = not significant; cv = coefficient of variation; na = not available.

	WEIGTH (CR)	TOTAL-BODY LENGTH (MM)	TAIL LENGTH (MM)	HINDFOOT LENGTH (MM)
Sicily	6.9 ± 1.16	69.8 ± 4.7	36.6 ± 3.74	12.3 ± 0.37
(unpubl. plus Sarà *et al.*, 1990)	(5.0-9.5); n=47	(63.0-79.0); n=24	(32.0-45.0); n=23	(11.5-13.0); n=24
cv	16.81%	6.73%	10.1%	3.01%
Egadi	6.1 ± 1.24	65.3 ± 3.6	33.3 ± 1.20	11.4 ± 0.34
(unpubl. **plus** Krapp, 1969)	(4.5-8.5); n=11	(61.5-71.5); n=9	(32.0-35.5); n=9	(11.0-12); n=8
cv	20.33%	5.51%	3.60%	3.258
Ustica	7.7 ± 1.15	72.0 ± 6.1	37.7 ± 1.53	12.2 ± 0.3
(unpubl.)	(7.0-9.0); n=3	(68.0-79.0); n=3	(36.0-39.0); n=3	(11.9-12.5); n=3
cv	14.94%	8.5%	4.0%	2.5%
Gozo	na	50.3	27.5	11.6
(Schembri and Schembri, 1976)		n =1	n =1	n =1
t Student Sicily vs Egadi	2.25 $p < 0.05$	7.71 $p < 0.001$	6.24 $p < 0.001$	2.75 $p = 0.01$
t Student Sicily vs Ustica	1.27 **p** = ns	2.33 $p < 0.05$	1.28 **p** = ns	0.20 **p** = ns
t Student Ustica vs Egadi	2.16 $p = 0.05$	6.42 $p < 0.001$	5.67 $p < 0.001$	1.41 **p** = ns

The body measurements of the three shrews from Ustica are larger than the Egadi specimens and were found to be significantly different, except for the hindfoot length. The Ustica specimens indicate the presence of a sligthly larger population not significantly different, except for the total body length, from the Sicilian shrews (cfr. min-max values in Tab. 4). The Ustica population shows thus a trend towards an increase of body size rather than a decrease as occurs in the Egadi (and in Gozo?). This result needs confirmation through the analysis of additional specimens, although the coefficients of variation (cfr. cv in Tab. 4) of the characters indicate a relative homogeneity of this small sample.

Some other external characters appear to be different in the Ustica shrews, the outer ear and the tail. This latter is slightly longer and more robust than the other *C. sicula* shrews.

The fur colour so far described in *C. sicula*, is very similar to that of *C. leucodon*

(Vogel, 1988). It is dark-grey or medium-grey on the dorsal side, and dirty white on the ventral side. Another typical characteristic is the white spot at the end of the tail. All the specimens so far trapped in Sicily (n > 50), Malta (n= 4) and Marettimo (n =15) show this pattern.

The color of the Ustica shrews is completely different from the other conspecifics populations, resembling more the C. *russula* fur, i.e. dark grey-brownish both on the dorsal and ventral side, and lacking the white spot at the end of the tail (Fig. 5).

Figure 5 The color **fur** of C. *sicula* from Ustica is different from that of other conspecific populations. From left **to** right: San Vito lo Capo (TP), Gratteri (Pa), Ustica, Ustica and Marettimo (Egadi).

DISCUSSION

The karyotype analysis proved the occurrence of a single taxon in all the checked islands and morphometrics suggest that the shrews from Favignana and Levanzo, still unkaryotyped, are also C. *sicula.*

The species is extinct in Malta and Lampedusa *(Sarh,* 1995) and apparently is lacking from the Eolian archipelago.

C. *sicula* shows a relatively high degree of geographic variation, if compared to its narrow geographic range. Every island has its own characteristics and morphometrics and this situation can be explained by an independent character divergence of insular populations as sustained by Thorpe (1987).

Most (76%) of this variation is, in the form of a size decrease; which is more o less pronounced in each island if compared to the 'morphotype' of the shrew living in Sicily. This latter presumably is the founder population. The skull and mandible size in the Egadi population and possibly the Gozo population, is found to decrease homogeneously to a smaller body size.

Selective forces determined by the shared micro-insularity conditions (relative semplicity of ecosystems, small physical size and area, climatic analogies, lacks of predators and competitors, etc.) can explain this common evolutive trend. In this case; insularity acts as a cohesive force balancing the genetic divergence due to different founder events and substituting the cohesive force of gene flow (cfr. Case, 1978; Davis, 1983). Sondaar (1977) indeed suggested that the evolution of insular Pleistocene Mammals was directional and thus driven by a strong selective pressure similar on different islands.

The populations of C. *sicula* around Sicily show a similar adaptive response, i.e. the size reduction and related shape changes, to similar selective pressures due to their life on a small island. This adaptive response is, however, independent and mediated by time and historical events, i.e. by the paleobiogeography of these islands (Sari, 1995).

The Egadi is a calcareous archipelago, which formed together with Sicily and the Maltese archipelago, **a** single island during the Upper Pleistocene (La Greca, 1961). The furthest Maltese archipelago and Marettimo island were the first to separate from Sicily at the end of the Wurmian glacial period. The populations of the islands long ago isolated from Sicily have undergone a higher degree of shape variation than those of the nearer Levanzo and Favignana (cfr. Figs 3, below and 4, below).

The Ustica population is a case apart, and its morphometrics and external phenotype are the most divergent from those typical of C. *sicula*. Melanism could have been an adaptation to this particular insular environment. Ustica is the only volcanic island within the C. *sicula* range. It emerged at least 1,000,000 years b.p. and has been always been separated from Sicily by a deep (max depth 2,000 m b.s.l.) and wide (67 **km)** sea channel.

The shrews' colonization of this island has probably been fairly recent, since its terrestrial fauna is characterized by recent immigrant taxa (Massa and Di Palma, 1988), whose occurrence was best explained by rafting or anthropogenic introduction during the late Neolithic (half of II millennium b. C.).

In conclusion, it can be assumed that the higher morphometric variation of this latter population, only recently isolated from its Sicilian counterpart, could have been caused by a genetic drift as well as by a directional selection stronger than in the other small calcareous islands.

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