

Distribution of genetic variation and taxonomy of insular and mainland populations of the Italian wall lizard, *Podarcis sicula*

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Abstract. The genetic structure and heterogeneity of *Podarcis sicula* (Reptilia, Lacertidae) was studied in insular (Pontine Archipelago) and mainland (central and southern Italy) populations by means of allozyme electrophoresis at 20 presumptive gene loci. Genetic variability in the species is low and genetic subdivision is high. The highest values of percent polymorphism and heterozygosity were found in the samples from the southernmost part of Italy (Calabria). The insular samples from the Pontine Archipelago were characterized by loss of alleles and erosion of genetic variability. Population heterogeneity analysis carried out by the estimation of Wright's *F*-statistics demonstrated substantial genetic differentiation among populations. *F*-statistics and genetic distance data show that genetic variation is distributed into three population groups. The first group includes the genetically very similar populations from central Italy and the Pontine Archipelago, the second includes the populations from Campania (southern Italy), the third comprises the populations from the southernmost part of the Italian Peninsula (Calabria). Based on the results of the allozyme data, the systematic status of the subspecies of *P. sicula* occurring in the studied areas is discussed.

Introduction

Podarcis sicula is a member of a lacertid genus that includes several species endemic to Mediterranean islands (Arnold, 1973). It is a polytypic species occurring in mainland Italy, Sicily, Sardinia, Corsica, in the coastal regions of Slovenia and Croatia, and in some areas of Montenegro (Lanza, 1968; Henle and Klaver, 1986). This lizard inhabits also most of the Italian islands and islets (see Corti and Lo Cascio, 2002, for an updated list of islands).

Podarcis sicula appears to be a successful colonizer, as it has been introduced and acclimatized to several extra-range localities. Naturalized populations are found in Spain (Almeria and Santander), Portugal (Lisbon), on Menorca Island (Balearic Islands), in France (Tolone and on the Château d'If Island), in Turkey (Istanbul and on some islands of the Marmara Sea), in North Africa (Tunis and Tripolis), and in the U.S.A. (Philadelphia, Topeka, Long Island) (Capula, 1994a; Oliverio et al., 2001; Corti and Lo Cascio, 2002).

The occurrence of this species on some Italian islands seems to be the result of human transportation followed by acclimatization as well (Lanza, 1973, 1983; Capula, 1992, 1994a, b; Lo Valvo and Nicolini, 2001). In some cases at least, it has been demonstrated that the introduced populations of *P. sicula* have competed successfully with the native *Podarcis* species, greatly reducing the range of these latter, as e.g. on the Aeolian Islands, where the native *P. raffonei* is presently confined to only one large island and three islets and is nearly reaching extinction (Capula, 1992; Capula et al., 2002). *Podarcis sicula*, when coming into contact with the genetically related species of the genus *Podarcis* (e.g. *P. melisellensis*, *P. raffonei*, *P. tiliguerta*, *P. wagleriana*), may also hybridize in narrow overlap zones, especially in insular Mediterranean habitats altered by human activities (Gorman et al., 1975; Capula, 1993, 2002).

These data clearly indicate that *P. sicula* is an opportunistic species characterized by broad ecological tolerance and high spreading capacity (Nevo et al., 1972; Gorman et al., 1975).

Podarcis sicula has high morphological and chromatic variability throughout its range (Arnold, 2002), and several insular subspecies have been described on a morphological basis (see Amori et al., 1993, and Corti and Lo Cascio, 2002, for an updated list), starting with the famous “blue lizard” (*P. s. coerulea*) from the Faraglioni Rocks near Capri Island (Tyrrhenian Sea, southern Italy), which was described by Eimer in 1872. However, the systematics of these intraspecific taxa is worthy of reconsideration and the existence of most of them could not be supported by allozyme or molecular data (Corti et al., 1989; Amori et al., 1993; Capula, 1994a; Oliverio et al., 2001). Evidence concerning the rapid morphological divergence after colonization of new islands by some species of iguanid lizards of the genus *Anolis* (Losos et al., 1997) would indicate that, especially in insular habitats, morphological and chromatic characters can change in a very short time span. It is therefore evident that the utilization of morphological characters of lizards for systematic purposes may be misleading, as these characters are highly related to climatic and environmental factors.

In this paper, based primarily on allozyme data, the distribution of genetic variation within and among insular and mainland populations of *P. sicula* was estimated. Moreover, in order to test whether the existence of some morphologically based subspecies is supported by genetic data, estimates of genetic differentiation among populations were provided.

Materials and methods

Sampling. Samples were obtained from eight mainland sites (Latium, central Italy; Campania and Calabria, southern Italy) and six insular localities (Pontine Islands, Tyrrhenian Sea) between May 1998 and September 2000 (fig. 1). For interspecific comparison one sample of the closely related species *P. muralis* from central Italy was also employed. The precise geographic origin of each sample, the number of specimens analysed per population and the subspecies studied are indicated in table 1.

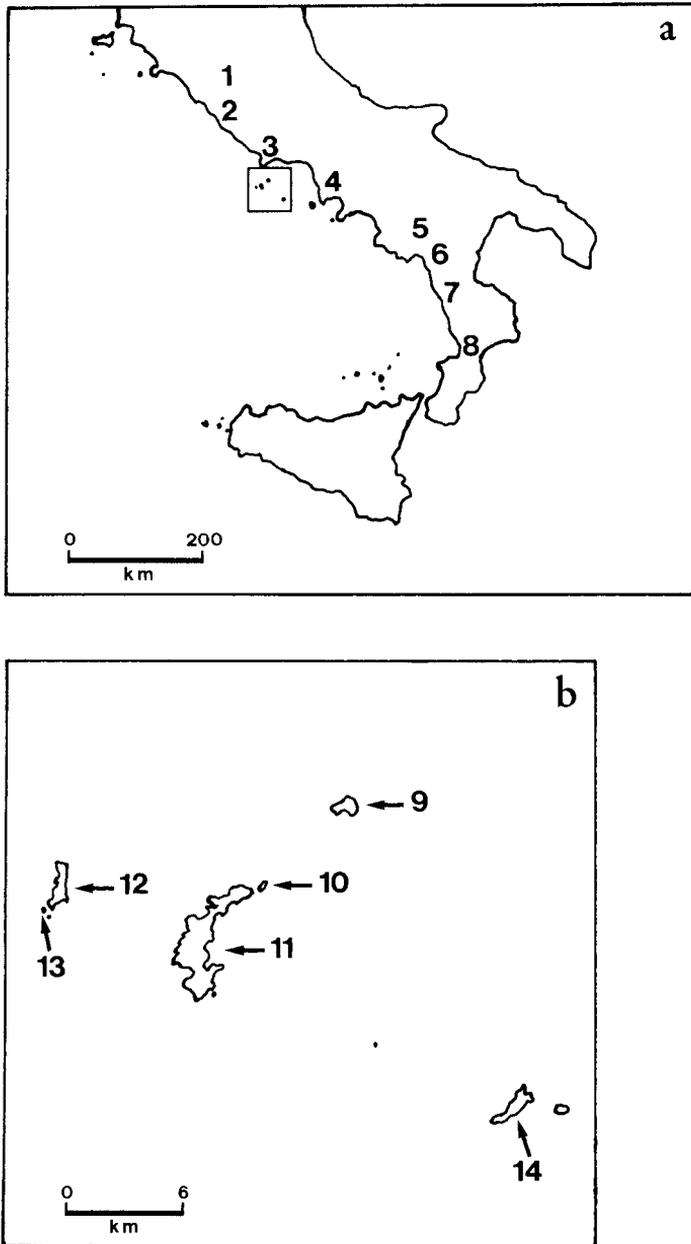


Figure 1. Maps of peninsular Italy (a) and the Pontine Archipelago (b) showing localities from which *P. sicula* samples were examined biochemically. a: 1, Castel Giuliano; 2, Ostia; 3, San Felice Circeo; 4, Napoli; 5, Rofrano; 6, Fagnano Castello; 7, Fiumefreddo Bruzio; 8, Catanzaro; b: 9, Zannone Island; 10, Gavi Islet; 11, Ponza Island; 12, Palmarola Island; 13, Scoglio Cappello Islet; 14, Ventotene Island. The open square in (a) indicates the position of the Pontine Archipelago.

Table 1. Geographic, taxonomic and collecting data for *Podarcis sicula* and *P. muralis* samples used in this study. The nomenclature of the subspecies is according to the current classification (Amori et al., 1993). For geographic localization of the *P. sicula* collecting localities see figure 1.

Population	Subspecies	Sample size	Locality
1	<i>Podarcis sicula campestris</i>	6	Castel Giuliano
2	<i>Podarcis sicula campestris</i>	6	Ostia
3	<i>Podarcis sicula campestris</i>	5	San Felice Circeo
4	<i>Podarcis sicula sicula</i>	10	Napoli
5	<i>Podarcis sicula sicula</i>	16	Rofrano
6	<i>Podarcis sicula sicula</i>	4	Fagnano Castello
7	<i>Podarcis sicula sicula</i>	10	Fiumefreddo Bruzio
8	<i>Podarcis sicula sicula</i>	6	Catanzaro
9	<i>Podarcis sicula patrizii</i>	3	Zannone Island, Pontine Archipelago
10	<i>Podarcis sicula lanzai</i>	3	Gavi Islet, Pontine Archipelago
11	<i>Podarcis sicula latastei</i>	8	Ponza Island, Pontine Archipelago
12	<i>Podarcis sicula palmarolae</i>	3	Palmarola Island, Pontine Archipelago
13	<i>Podarcis sicula pasquinii</i>	2	Scoglio Cappello Islet, near Palmarola Island, Pontine Archipelago
14	<i>Podarcis sicula sicula</i>	2	Ventotene Island, Pontine Archipelago
15	<i>Podarcis muralis brueggemanni</i>	15	Firenze

To avoid killing animals or injurious biopsy, approximately 1 cm of the tail of each lizard was taken off following the suggestion of Mayer and Tiedemann (1985). After collecting the piece of tail, lizards were sexed and then released to the site where they were collected. The tail fragment was kept in Eppendorf reaction tubes (2 mL), and stored below -70°C until electrophoretic analysis.

Electrophoresis. The electrophoretic analysis was undertaken for 99 specimens from all 15 localities. Standard horizontal starch gel electrophoresis was performed on tail muscle tissue; parts of this tissue were crushed in 0.1 mL of distilled water. Gene products for the following 20 presumptive enzyme loci were analysed: glycerol-3-phosphate dehydrogenase (E.C. 1.1.1.8, *α Gpd*), lactate dehydrogenase (E.C. 1.1.1.27, *Ldh-1*, *Ldh-2*), malate dehydrogenase (E.C. 1.1.1.37, *Mdh-1*, *Mdh-2*), malic enzyme (E.C. 1.1.1.40, *Me-1*, *Me-2*), isocitrate dehydrogenase (E.C. 1.1.1.42, *Idh-1*, *Idh-2*), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44, *6Pgd*), glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12, *Gapd*), superoxide dismutase (E.C. 1.15.1.1, *Sod-1*), glutamate-oxaloacetate transaminase (E.C. 2.6.1.1, *Got-1*, *Got-2*), creatine kinase (E.C. 2.7.3.2, *Ck*), adenylate kinase (E.C. 2.7.4.3, *Ak*), mannose-6-phosphate isomerase (E.C. 5.3.1.8, *Mpi*), glucose-6-phosphate isomerase (E.C. 5.3.1.9, *Gpi*), phosphoglucomutase (E.C. 5.4.2.2, *Pgm-1*, *Pgm-2*). The buffer systems used, electrophoretic procedures, staining techniques, and loci and allele designations were those described by Capula (1990, 1994b).

Analysis. Genotypic and allele frequencies were determined by direct count from allozyme phenotypes, and the resulting data were analysed by various statistical methods to describe the genetic structure of the *P. sicula* populations. Genotypic proportions expected on the basis of Hardy-Weinberg equilibrium were calculated by Levene's (1949) formula for small samples. The statistical significance of departures from Hardy-Weinberg equilibrium was estimated using a test for calculating exact significance probabilities (Haldane, 1954; Elston and Forthofer, 1977). To determine whether the heterogeneity in the genotypic distribution reflects differences in allele frequencies, the variation in genic proportions among populations was subjected to a contingency χ^2 analysis (Workman and Niswander, 1970). The genetic variability for each population and for the species as a whole was estimated using the following parameters: mean number of alleles per locus (A), percentage of loci polymorphic (P , at the 99% level), observed mean heterozygosity (H_o), expected mean heterozygosity (H_e) in Hardy-Weinberg equilibrium (unbiased estimate; Nei, 1978).

The distribution of genetic variation within and among populations was assessed using Wright's F -statistics (Wright, 1965, 1978). F_{ST} denotes the level of substructuring within the total population and ranges from

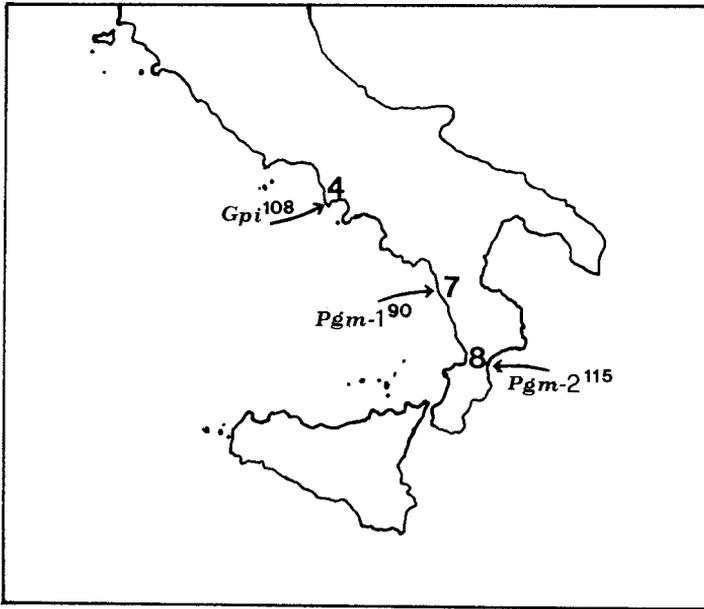


Figure 2. Alleles specific to the *Podarcis sicula* populations from the southern Italian Peninsula (4 = Napoli; 7 = Fiumefreddo Bruzio; 8 = Catanzaro).

Me-1, *6Pgd*, *Mpi*), and three loci were local (<3 populations) and weakly polymorphic (*Gpi*, *Pgm-1*, *Pgm-2*). The samples from the Pontine Islands (Zannone, Gavi, Ponza, Palmarola, Scoglio Cappello, Ventotene) had no unique alleles (sensu Slatkin, 1987) and were characterized by a predominance of fixed alleles at each locus. A similar situation was found in the samples from the central Italian Peninsula (Castel Giuliano, Ostia, San Felice Circeo). On the other hand, three out of the five samples from the southern Italian Peninsula (Napoli, Fiumefreddo Bruzio, Catanzaro) showed three unique alleles at the *Gpi*, *Pgm-1* and *Pgm-2* loci respectively (fig. 2). The total number of alleles (t.n.a.) in the eight samples from mainland Italy was higher (average t.n.a. = 23.12) than that found in the insular samples (average t.n.a. = 20.83), and the difference was statistically significant ($P < 0.05$, *t*-test).

The results of the contingency χ^2 analysis are given in table 3. This analysis reveals that five out of seven variable loci exhibit statistically significant heterogeneity in the allele frequencies. This result indicates that there are significant differences among the gene pools of the studied populations, suggesting local genetic differentiation.

Significant deviations from Hardy-Weinberg equilibrium in the direction of heterozygote deficiencies were found for the following populations and loci (in parentheses): Napoli (*Gpi*, $P < 0.01$), and Catanzaro (*Me-1*, $P < 0.01$).

The estimated measures of genetic variability are given in table 4. The overall mean number of alleles per locus was 1.09, ranging from 1.0 to 1.3. The proportion of

Table 3. Chi-square values resulting from contingency χ^2 analysis at the polymorphic loci among populations of *Podarcis sicula*. d.f. = degrees of freedom; NS, nonsignificant.

Locus	No. of alleles	χ^2	d.f.	<i>P</i>
<i>Ldh-1</i>	2	114.151	13	<0.001
<i>Me-1</i>	3	82.019	26	<0.001
<i>6Pgd</i>	2	49.043	13	<0.001
<i>Mpi</i>	2	89.406	13	<0.001
<i>Gpi</i>	3	127.385	26	<0.001
<i>Pgm-1</i>	2	10.637	13	NS
<i>Pgm-2</i>	2	13.078	13	NS
Total		485.718	117	<0.001

Table 4. Genetic variability parameters in *Podarcis sicula* populations. *A*, mean number of alleles per locus; *P*, mean proportion of polymorphic loci; *H_o*, observed mean heterozygosity; *H_e*, expected mean heterozygosity; (*s_x*), standard error.

Population	<i>A</i>	<i>P</i>	<i>H_o</i>	(<i>s_x</i>)	<i>H_e</i>	(<i>s_x</i>)
1. Castel Giuliano	1.1	15	0.033	0.019	0.032	0.018
2. Ostia	1.0	5	0.013	0.013	0.013	0.013
3. San Felice Circeo	1.0	0.0	0.000	0.000	0.000	0.000
4. Napoli	1.1	10	0.025	0.018	0.031	0.023
5. Rofrano	1.2	20	0.052	0.028	0.059	0.033
6. Fagnano Castello	1.1	10	0.063	0.044	0.055	0.038
7. Fiumefreddo Bruzio	1.3	25	0.071	0.032	0.082	0.035
8. Catanzaro	1.3	25	0.050	0.021	0.080	0.036
9. Zannone Island	1.1	10	0.033	0.023	0.047	0.034
10. Gavi Islet	1.0	0.0	0.000	0.000	0.000	0.000
11. Ponza Island	1.0	5	0.013	0.012	0.025	0.025
12. Palmarola Island	1.0	5	0.017	0.017	0.017	0.017
13. Scoglio Cappello Islet	1.0	5	0.025	0.025	0.025	0.025
14. Ventotene Island	1.0	0.0	0.000	0.000	0.000	0.000

polymorphic loci (*P*) ranged from 0.0 (San Felice Circeo, Gavi, Ventotene) to 0.25 (Fiumefreddo Bruzio, Catanzaro), averaging 0.10. The observed heterozygosity (*H_o*) showed a similar trend, ranging from 0.000 (San Felice Circeo, Gavi, Ventotene) to 0.071 (Fiumefreddo Bruzio) and averaging 0.029. The samples from mainland Italy showed values of average polymorphism and heterozygosity (*P* = 0.14; *H_o* = 0.038) relatively higher than those detected in the samples from the Pontine Archipelago (*P* = 0.04; *H_o* = 0.015) and the difference was statistically significant (*P* < 0.05, *t*-test). For 4 populations (Fiumefreddo Bruzio, Catanzaro, Zannone, Ponza) heterozygosity was slightly lower than that expected under Hardy-Weinberg equilibrium (table 4).

Table 5 provides estimates of *F_{IS}*, *F_{IT}* and *F_{ST}* for all variable loci among *P. sicula* samples. The seven polymorphic loci evaluated for all populations yielded low *F_{IS}* values, and mean *F_{IS}* was low as well (0.060). This is probably because Hardy-Weinberg proportions are maintained within populations by random mating. The weighted mean

Table 5. Summary of F -statistics at seven variable loci for *Podarcis sicula* populations. Asterisk denotes statistical significance as determined by the χ^2 test (see text). * $P < 0.0001$; NS = not significant.

Locus	F_{IS}	F_{IT}	F_{ST}
<i>Ldh-1</i>	-0.399	0.618	0.727*
<i>Me-1</i>	0.150	0.420	0.317*
<i>6Pgd</i>	-0.025	0.221	0.241*
<i>Mpi</i>	0.338	0.687	0.526*
<i>Gpi</i>	0.152	0.725	0.676*
<i>Pgm-1</i>	-0.077	-0.005	0.067 NS
<i>Pgm-2</i>	-0.091	-0.006	0.078 NS
Mean	0.060	0.509	0.478

value for F_{IT} was 0.509, indicating a slight heterozygote deficiency within the species. F_{ST} values ranged from 0.067 (*Pgm-1*) to 0.727 (*Ldh-1*) and were high for the loci *Ldh-1*, *Mpi* and *Gpi*. Five of the 7 single locus F_{ST} values were statistically significant, suggesting a certain amount of genetic differentiation among populations. The weighted mean value of F_{ST} was 0.478, indicating that 48 per cent of genetic variation in *P. sicula* is attributable to differentiation among populations.

The values of standard genetic identity and genetic distance for each pairwise comparison are given in table 6. Nei's standard genetic distance (D) between the *P. sicula* populations ranged from 0 (between S. Felice Circeo and Gavi, between S. Felice Circeo and Ventotene, and between Ventotene and Gavi) to 0.106 (between Napoli and Fiumefreddo Bruzio), averaging 0.031 across all populations. Surprisingly, despite the geographic distance and the wide sea channel separating the Pontine Archipelago from central Italy, the samples from these areas were genetically quite similar to each other (average $D = 0.005$). Very low values of standard genetic distance were also found (i) among the Pontine Islands (average $D = 0.006$), which are inhabited by five endemic subspecies (see table 1), and (ii) within central Italy (average $D = 0.002$). On the other hand, higher values of genetic distance were found comparing the samples from Calabria (Fagnano Castello, Fiumefreddo Bruzio, Catanzaro) with (i) those from central Italy (average $D = 0.051$), and (ii) those from the Pontine Archipelago (average $D = 0.055$). The comparison between the samples from Calabria and Campania (Napoli, Rofrano) gave the highest distances (average $D = 0.086$). This clearly indicates that the *P. sicula* populations occurring in the southern Italian Peninsula are genetically differentiated to each other and from those inhabiting central Italy and the Pontine Archipelago.

As to the interspecific genetic distances, the comparison between *P. sicula* and *P. muralis* gave a relatively low value of Nei's genetic distance (average $D = 0.157$), although falling into the range obtained from comparisons between well recognized biological species of the genus *Podarcis*. This indicates that the two species are genetically closely related.

The genetic relationships among the samples studied are presented in figure 3. The UPGMA clustering procedure revealed five main clusters in the phenogram constructed on

Table 6. Values of Nei's (1972) standard genetic identity (above the diagonal) and standard genetic distance (below the diagonal) among populations of *Podarcis sicula* and *P. muralis*. For geographical origin of populations see table 1.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	–	0.998	0.998	0.965	0.967	0.969	0.942	0.949	0.989	0.998	0.994	0.998	0.997	0.998	0.875
2	0.002	–	0.999	0.966	0.965	0.966	0.936	0.945	0.987	0.999	0.992	0.998	0.999	0.999	0.865
3	0.002	0.001	–	0.967	0.959	0.967	0.934	0.944	0.986	1.000	0.993	0.999	0.997	1.000	0.867
4	0.036	0.035	0.034	–	0.938	0.933	0.899	0.910	0.957	0.967	0.963	0.967	0.963	0.967	0.836
5	0.034	0.036	0.042	0.064	–	0.925	0.915	0.924	0.989	0.959	0.978	0.969	0.970	0.959	0.855
6	0.032	0.034	0.033	0.070	0.077	–	0.990	0.991	0.952	0.967	0.960	0.966	0.964	0.967	0.830
7	0.060	0.066	0.068	0.106	0.089	0.010	–	0.989	0.928	0.934	0.930	0.934	0.937	0.934	0.804
8	0.052	0.057	0.058	0.095	0.080	0.009	0.011	–	0.939	0.944	0.942	0.945	0.944	0.944	0.821
9	0.011	0.013	0.014	0.044	0.011	0.049	0.075	0.063	–	0.986	0.998	0.993	0.987	0.986	0.875
10	0.002	0.001	0.000	0.034	0.042	0.033	0.068	0.058	0.014	–	0.993	0.999	0.997	1.000	0.867
11	0.006	0.008	0.007	0.038	0.023	0.041	0.073	0.060	0.002	0.007	–	0.998	0.990	0.993	0.876
12	0.002	0.002	0.001	0.034	0.032	0.035	0.068	0.057	0.007	0.001	0.002	–	0.995	0.999	0.873
13	0.003	0.001	0.003	0.038	0.031	0.037	0.065	0.058	0.013	0.003	0.010	0.005	–	0.997	0.862
14	0.002	0.001	0.000	0.034	0.042	0.033	0.068	0.058	0.014	0.000	0.007	0.001	0.003	–	0.867
15	0.134	0.145	0.143	0.180	0.156	0.186	0.218	0.198	0.134	0.143	0.132	0.136	0.148	0.143	–

the basis of the matrix of Nei's standard genetic distances. Within the first cluster, which includes the samples from central Italy and the Pontine Archipelago, the existence of two subclusters should be noted. The first subcluster includes the closely grouped samples from central Italy (Castel Giuliano, Ostia, San Felice Circeo) and four Pontine islands (Gavi, Palmarola, Scoglio Cappello and Ventotene), the second includes the samples from the other two Pontine islands, i.e. Zannone and Ponza. The second cluster contains only the sample from Rofrano, and the third cluster includes the sample from Napoli. The fourth cluster contains three subclusters corresponding to the samples from Calabria (Fagnano Castello, Catanzaro and Fiumefreddo Bruzio respectively). The fifth cluster contains the only one population of *P. muralis*. Cophenetic correlation between the matrix of genetic distances and the derived phenogram was rather high (0.957).

Discussion

The results of the allozyme analyses indicate that genetic variability is relatively low in *P. sicula*. The Italian wall lizard shows values of polymorphism and heterozygosity lower than (i) those detected in the phylogenetically related *P. wagneriana* from Sicily ($P = 0.15$; $H_o = 0.037$; Capula, 1994b), (ii) the average ones calculated by Capula (1990) for nine species of the genus *Podarcis* ($P = 0.13$; $H_o = 0.053$), and (iii) the average ones calculated by Nevo (1978) for 17 species of reptiles ($P = 0.22$; $H_o = 0.047$). The highest values of heterozygosity were found in the samples from southern Italy, whereas the lowest ones were observed in some samples from central Italy (San Felice Circeo) and the Pontine Archipelago (Gavi, Ventotene). All samples from the Pontine Islands were

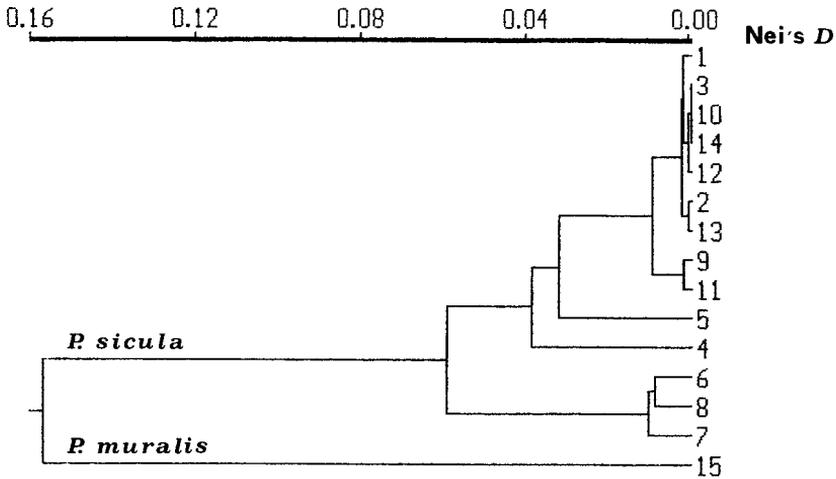


Figure 3. Phenogram generated by UPGMA cluster analysis based on Nei's (1972) standard genetic distances among *Podarcis sicula* and *P. muralis* populations from peninsular Italy and the Pontine Archipelago. For geographical origin of populations see table 1.

characterized by loss of alleles and erosion of genetic variability. This pattern could be caused by genetic drift phenomena, such as population bottlenecks (e.g. founder effect: the original colonizers carry only a subsample of the genetic variability of the parental population; Selander, 1976), as supported by the fact that these populations (i) have no unique alleles, (ii) are characterized by a predominance of fixed alleles at each locus, (iii) are genetically very similar to each other ($D = 0.006$) and to the geographically closest populations from central Italy ($D = 0.005$). The analysis of allele frequencies and the genetic variability and genetic distance data suggest that (i) the occurrence of *P. sicula* on these islands could be the result of human transportation followed by acclimatization, and (ii) the Pontine Archipelago populations were probably founded by a small number of individuals accidentally introduced, presumably from central Italy, in historical or proto-historical times.

The genetic heterogeneity analysis demonstrates a certain amount of genetic differentiation among local populations of *P. sicula*, with a relatively high level of genetic subdivision. The estimated standardized variance in gene frequency (F_{ST}) for the total sample is highly significant, with a value (0.478) much higher than that calculated by Capula (1994b) for the phylogenetically related *P. wagleriana* from Sicily ($F_{ST} = 0.153$), and very similar to that found in *P. tiliguerta* from Corsica and Sardinia ($F_{ST} = 0.460$) (Capula, 1996). This indicates that 48 per cent of the gene diversity was between populations, and 52 per cent was within populations.

Allele frequencies data, F -statistics and genetic distance data show that, at the scale of the study, genetic variation in *P. sicula* is distributed into three main population groups. The first group includes the genetically very similar populations from central Italy and the

Pontine Archipelago; the second group includes the populations from Campania; the third group comprises the populations from Calabria. Genetic distance values found between the three population groups are relatively high (see table 6), although falling below those normally encountered comparing populations of well recognized biological species of the genus *Podarcis* (see e.g. Mayer, 1981; Thorpe, 1983; Capula, 1994a, b, c, 1996, 2003). These data are in agreement with the results of the molecular investigations (analysis of mitochondrial DNA sequences) carried out by Oliverio et al. (1998, 2001), which indicate a certain amount of molecular divergence between the *P. sicula* populations from central and southern Italy.

Based on these evidences, the existence of at least two subspecies appears to be supported by genetic data: *P. s. sicula* and *P. s. campestris*. Taking also into account the results of the allozyme analyses carried out by Capula (1990, 1994a), the populations from central and northern Italy, Corsica, the Tuscan Archipelago and the Pontine Islands should be ascribed to *P. s. campestris*; the populations from the southernmost part of the Italian Peninsula (Calabria) should be ascribed to *P. s. sicula*. It is therefore evident that the morphologically based subspecies endemic to the Pontine Islands (*lanzai*, *latastei*, *palmarolae*, *pasquini*, *patrizii*; see table 1) should be synonymized with *P. s. campestris*. As to the populations from Campania, these were attributed by Taddei (1949) to the subspecies *P. s. campana* on morphological basis, but subsequently this intraspecific taxon was synonymized with *P. s. sicula* (Mertens and Wermuth, 1960; Lanza, 1968). According to the results of the present study, the populations from Campania are genetically differentiated from those ascribed to *P. s. sicula* and also from those ascribed to *P. s. campestris*. These populations should be therefore attributed to a distinct subspecies, for which the most appropriate name seems to be *campana*. A definitive assessment of the taxonomic status of the *P. sicula* populations inhabiting Campania and other neighbouring areas of southern Italy, i.e. Molise, Puglia and Basilicata, will probably require further genetic investigations as well as the analysis of a larger number of samples from the southern Italian Peninsula.

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