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Population genetics of a colonizing lizard: loss of variability in introduced populations of *Podarcis sicula*

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Abstract. Allozyme electrophoresis was used to study the genetic variability (proportion of polymorphic loci and heterozygosity) in insular populations (Corsica, Elba, Montecristo, Marettimo, Pantelleria) of the lacertid lizard *Podarcis sicula*. These populations were presumed to have originated from episodes of accidental anthropogenic introduction. In order to test the hypothesis of a man-aided colonization and to provide comparative data, heterozygosity and polymorphism were also estimated in autochthonous populations of *P. sicula* from the Italian peninsula and Sicily. In each case, the presumed introduced population showed levels of genetic variability significantly lower than those detected in the autochthonous ones. Very little genetic differentiation was found among native and presumed colonist populations, Nei's standard genetic distances ranging from 0.001 to 0.009. These results strongly support the hypothesis that *P. sicula* was only recently introduced to the studied islands, and provide additional evidence of reduced genetic variability due to founder effect in insular populations originating from episodes of human transportation.
Key words. Allozyme electrophoresis; genetic variability; genetic differentiation; island colonization; *Podarcis sicula*; Reptilia; Sauria; Lacertidae.

In the past 20 years, electrophoretic surveys of island species have been helpful in studying the genetics of the colonization process[1–5]. According to the data provided in previous studies[6,7], the colonization of an island by a new species should be accompanied by severe reduction of genetic variability in the founding populations. The major causes of the reduced variability of colonizing populations are the founder effect (the original colonizers carry only a subsample of the genetic variability of the parental population) and intermittent random drift, but fixation of alleles has also been attributed to directional selection[8–10]. Examples of reduced genetic variability determined by allozyme electrophoresis have been documented in insects[11–13], land snails[14], elephant seals[15], and lizards[7,16].

The aim of the present study was to investigate allozyme variation in mainland and insular populations of the lacertid lizard *Podarcis sicula* by means of multilocus electrophoresis. *Podarcis sicula* occurs as autochthonous species in peninsular Italy, Sicily and in a number of Tyrrhenian islands[17]. This lizard appears to be a successful colonizer[18,19], as it has been introduced and acclimatized to several extra-range localities [e.g., Almeria (S.E. Spain), Minorca Island (Balearic Islands), Île du Chateau d'If (Marseille, S. France), Sea of Marmara area (European Turkey), Philadelphia (Pennsylvania, USA)][20]. According to previous studies[21–24], the occurrence of the species on some Mediterranean islands, i.e. Corsica, Elba, Montecristo, Marettimo, and Pantelleria, could be the result of human transportation followed by acclimatization as well. However, none of the authors who suggested the hypothesis of a man-aided colonization was able to find compelling circumstantial evidence. In order to test this hypothesis, the genetic variability parameters of the presumed introduced populations were compared with those of native populations from peninsular Italy and Sicily, and estimates of genetic differentiation among populations were provided.

Materials and methods

Animals. Samples of *Podarcis sicula* used in this study were obtained from 10 localities, including the Italian peninsula (1, Firenze; 2, Monte Argentario; 3, Napoli), three Tyrrhenian islands (4, Corsica (Caporalino); 5, Elba; 6, Montecristo), Sicily (7, Milazzo; 8, Selinunte), and two Sicilian islands (9, Marettimo; 10, Pantelleria). Since some of the genetic variability parameters estimated in this study (e.g. proportion of polymorphic loci) are highly dependent on sample size, the number of individuals in each sample was nearly identical (each sample was composed of ten individuals, excluding the samples from Milazzo, $N = 11$, and Marettimo, $N = 12$). To avoid animal killing and harmful biopsy, approximately 1 cm of the tail of each lizard was taken off following the suggestion by Mayer and Tiedemann[25]. The piece of tail was then kept in Eppendorf reaction tubes (2 ml) and stored below -70 °C until electrophoretic analysis.

Electrophoresis. Standard horizontal starch gel electrophoresis was performed on tail muscle tissue, which was crushed in distilled water. Homogenates from single individuals were absorbed into 5 × 5 mm pieces of chro-

matography paper (Whatman 3 MM) and inserted into a 10% Connaught starch gel. Electrophoresis was carried out at 7–9 V/cm for 4–6 h at 5 °C. After the run, gels were sliced into two parts and each slice was stained for a specific enzyme. Gene products for the following 21 presumptive enzyme loci were analyzed: *αGpd, Ldh-1, Ldh-2, Mdh-1, Mdh-2, Me-1, Me-2, Idh-1, Idh-2, 6Pgd, Gapd, Sod, Got-1, Got-2, Ck, Ak, Pgm-1, Pgm-2, Ca-2, Mpi, Gpi*. The buffer systems used, electrophoretic procedures, and staining techniques were those described by Capula[26,27]. The following loci and allele designations were adopted: isozymes were numbered in order of decreasing mobility from the most anodal; allozymes were named numerically according to their mobility relative to the commonest one found in a reference population of *P. sicula* from Napoli, indicated as 100 (>100 = faster mobility; <100 = slower mobility).

Analysis. The genetic variability of populations was estimated using the following parameters: mean number of alleles per locus (A); proportion of polymorphic loci, at the 99% level (P); observed mean heterozygosity per locus (H_o); expected mean heterozygosity per locus (H_e) (unbiased estimate[28]). The genetic relationships among the studied populations were evaluated using Nei's[29] standard genetic distance and Rogers'[30] genetic distance. All genetic variability and genetic distance measures were calculated by the computer program BIOSYS-1[31]. Estimation of phenetic relationships among populations was obtained by generating a phenogram of all samples by means of the unweighted pair-group method with arithmetic averaging (UPGMA)[32], based on the matrix of Rogers' genetic distances which are metrics (Nei's genetic distance measure is not a metric, i.e. violates the triangle inequality, and thus cannot be interpreted as a measure of evolutionary path length[33]).

Results

Of the 21 electrophoretic enzyme loci analyzed, eleven were found to be monomorphic and fixed for the same allele in all the studied samples (*αGpd, Ldh-2, Mdh-1, Me-2, Idh-1, Gapd, Sod, Got-1, Got-2, Ak, Ca-2*). The allele frequencies at the other 10 variable loci are given in table 1. The populations presumed to have been introduced (Corsica, Elba, Montecristo, Marettimo, Pantelleria) had no unique alleles, i.e. alleles found in only one population, and were characterized by a predominance of fixed alleles at each locus. Conversely, the samples from the Italian peninsula and Sicily showed some alleles which were not found ($Ldh\text{-}1^{115}$, $Mdh\text{-}2^{90}$, $Me\text{-}1^{92}$, $Idh\text{-}2^{96}$, $6Pgd^{95}$, $Pgm\text{-}2^{115}$, Mpi^{96}, Gpi^{90}) or were detected at low frequencies (e.g. $Pgm\text{-}1^{90}$) in the introduced populations. The total number of alleles (t.a.) detected in the native populations was therefore higher (average t.a. = 26) than that found in the five samples from the Tyrrhenian and Sicilian islands (average t.a. = 22).

The genetic variability parameters considered (A, P, H_o, H_e) are reported in table 2. The populations presumed to have been introduced showed levels of genetic variability noticeably lower than those found in the samples from peninsular Italy and Sicily. In the case of the Tyrrhenian islands (Corsica, Elba, Montecristo), the polymorphism was 28% of that found in the Italian peninsula (average $P = 4.8\%$ vs. average $P = 17.4\%$), while the observed heterozygosity was only 22% of that detected in the samples from central Italy (average $H_0 = 0.008$ vs. average $H_0 = 0.036$) (see fig. 1). In the case of Pantelleria and Marettimo islands the decrease in polymorphism was strong as well (Marettimo and Pantelleria: average $P = 9.5\%$; Sicily: average $P = 23.8\%$), but the observed heterozygosity showed a more dramatic reduction (Marettimo and Pantelleria: average $H_0 = 0.013$; Sicily: average $H_0 = 0.065$).

The values of genetic distance for each pairwise comparison are given in table 3. As would be expected both on the basis of the analysis of allele frequencies and the hypothesis discussed in the introduction, there was very little genetic differentiation between the presumed colonist populations and the native ones. In fact, Nei's D ranged from 0.001 to 0.003 (average $D = 0.002$) between the samples from the Tyrrhenian islands and those from the Italian peninsula, and from 0.007 to 0.009 (average $D = 0.009$) between the samples from Pantelleria and Marettimo and those from Sicily. Nei's genetic distances were particularly low 1) between the samples from peninsular Italy and those from Pantelleria and Marettimo (average $D = 0.002$), 2) among the samples from Corsica, Elba, and Montecristo (average $D = 0.001$), and 3) between the samples from Pantelleria and Marettimo (average $D = 0.001$).

The results of the UPGMA clustering procedure are shown in figure 2. Three main clusters are apparent in the phenogram constructed on the basis of the matrix of Rogers' genetic distances. Within the first cluster note the existence of three subclusters, one containing the populations from Firenze and Monte Argentario, one including the closely grouped populations from the Tyrrhenian islands and the Sicilian islands, and one containing the population from Napoli. The second cluster contains only the sample from Milazzo, and the third cluster includes the sample from Selinunte. This would suggest that 1) the populations occurring in the Italian peninsula are relatively differentiated from those inhabiting Sicily, 2) the populations from the Tyrrhenian islands (Corsica, Elba, Montecristo) are genetically very similar to those inhabiting the Italian peninsula and the Sicilian islands (Marettimo, Pantelleria), and 3) the populations from Pantelleria and Marettimo may have originated from individuals accidentally introduced from peninsular Italy. However, it is to be noticed that caution should be taken when interpreting genetic relationships among populations clustering at

Table 1. Allele frequencies at 10 variable loci in populations of *Podarcis sicula*. For geographic origin of populations see 'Materials and methods'. N, number of individuals scored per locus

Locus	Allele	Population									
		Mainland			Island			Mainland		Island	
		1	2	3	4	5	6	7	8	9	10
Ldh-1	(N)	10	10	10	10	10	10	11	10	12	6
	80	0.000	0.000	0.050	0.000	0.000	0.000	0.091	0.050	0.042	0.083
	100	1.000	1.000	0.950	1.000	1.000	1.000	0.864	0.950	0.958	0.917
	115	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000
Mdh-2	(N)	10	10	10	10	10	10	9	10	12	10
	90	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.000	0.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	0.944	1.000	1.000	1.000
Me-1	(N)	10	10	10	10	10	10	11	10	12	10
	92	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	96	0.100	0.150	0.000	0.200	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.900	0.850	0.900	0.800	1.000	0.900	0.591	1.000	1.000	1.000
	104	0.000	0.000	0.000	0.000	0.000	0.100	0.409	0.000	0.000	0.000
Idh-2	(N)	10	10	10	10	10	10	11	10	12	10
	96	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6Pgd	(N)	10	10	10	10	10	10	11	10	6	10
	95	0.100	0.100	0.100	0.000	0.000	0.000	0.000	0.050	0.000	0.000
	100	0.900	0.900	0.900	1.000	0.950	1.000	1.000	0.900	1.000	1.000
	105	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.050	0.000	0.000
Ck	(N)	10	10	10	10	10	10	7	10	12	8
	100	0.900	1.000	1.000	1.000	1.000	1.000	0.929	0.950	0.917	1.000
	110	0.100	0.000	0.000	0.000	0.000	0.000	0.071	0.050	0.083	0.000
Pgm-1	(N)	10	10	10	10	10	10	11	10	6	7
	90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.300	0.000	0.071
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.700	1.000	0.929
Pgm-2	(N)	10	10	10	10	10	10	11	10	12	7
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.700	1.000	1.000
	115	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.300	0.000	0.000
Mpi	(N)	6	10	10	6	10	6	11	10	12	8
	96	0.000	0.150	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000
	100	1.000	0.850	1.000	1.000	1.000	1.000	0.909	1.000	1.000	1.000
Gpi	(N)	5	10	10	10	10	10	11	10	12	10
	90	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	0.850	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	108	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2. Genetic variability parameters in the studied populations of *Podarcis sicula*. Mssl, mean sample size per locus; A, mean number of alleles per locus; P, mean proportion of polymorphic loci; H_o, observed mean heterozygosity; H_e, expected mean heterozygosity (unbiased estimate) (SE, standard error).

Genetic variability parameters	Population									
	Mainland			Island			Mainland		Island	
	1	2	3	4	5	6	7	8	9	10
Mssl	9.3	9.8	9.8	9.8	10.0	9.8	9.4	9.9	10.4	8.4
A	1.2	1.1	1.2	1.0	1.0	1.0	1.3	1.3	1.1	1.1
P	19.0	14.3	19.0	4.8	4.8	4.8	23.8	23.8	9.5	9.5
H_o	0.033	0.038	0.038	0.010	0.005	0.010	0.055	0.076	0.012	0.015
(SE)	(0.016)	(0.021)	(0.019)	(0.010)	(0.005)	(0.010)	(0.026)	(0.040)	(0.009)	(0.010)
H_e	0.032	0.035	0.036	0.016	0.005	0.009	0.057	0.061	0.012	0.015
(SE)	(0.015)	(0.019)	(0.018)	(0.016)	(0.005)	(0.009)	(0.027)	(0.030)	(0.008)	(0.010)

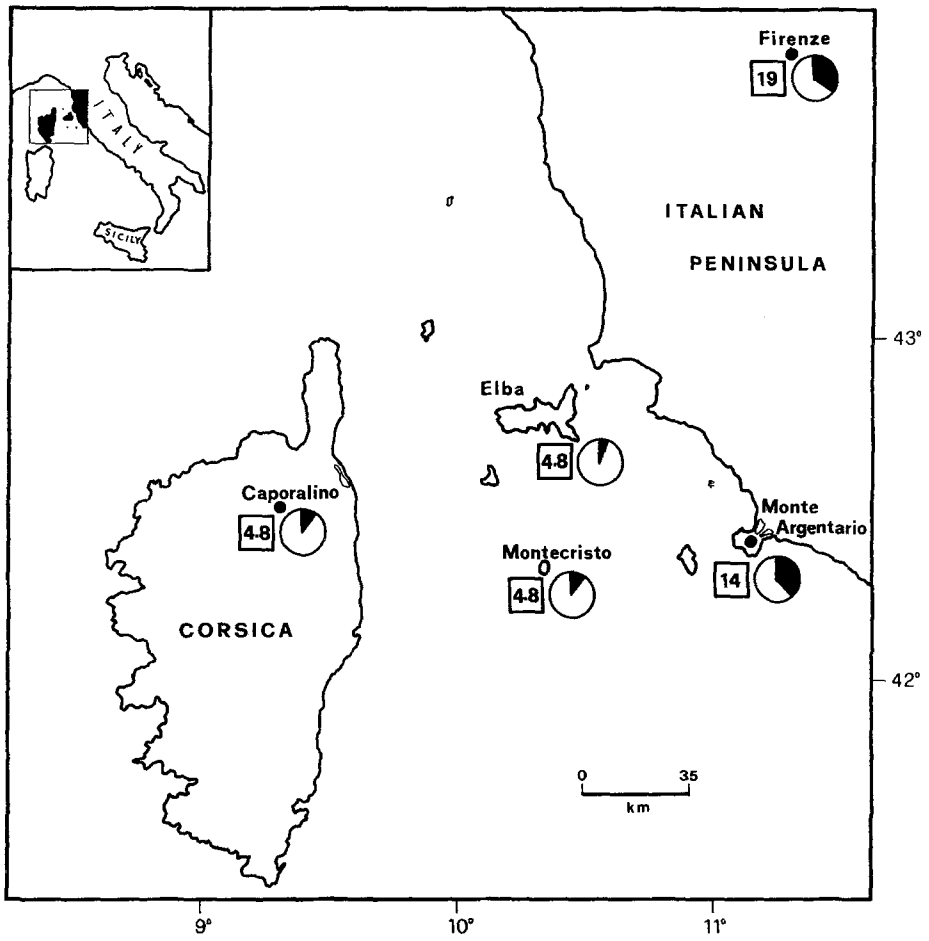

Figure 1. Comparison of genetic variability parameters in populations of *Podarcis sicula* from the Italian peninsula and Tyrrhenian islands. Black areas of pie diagrams represent percent observed heterozygosity per population (complete black pie would represent 10%). Squares indicate percent of polymorphic loci per population.

Table 3. Values of Nei's standard genetic distance (below the diagonal) and Rogers' genetic distance (above the diagonal) among populations of *Podarcis sicula*. For geographic origin of population see 'Materials and methods'.

Population	1	2	3	4	5	6	7	8	9	10
1	-	0.017	0.021	0.017	0.016	0.017	0.039	0.043	0.015	0.024
2	0.002	-	0.022	0.014	0.018	0.018	0.036	0.050	0.025	0.026
3	0.002	0.003	-	0.022	0.018	0.018	0.043	0.044	0.020	0.021
4	0.002	0.002	0.003	-	0.012	0.008	0.033	0.047	0.015	0.017
5	0.001	0.002	0.002	0.002	-	0.007	0.038	0.036	0.008	0.010
6	0.002	0.002	0.002	0.001	0.001	-	0.031	0.042	0.011	0.012
7	0.009	0.008	0.009	0.008	0.010	0.006	-	0.064	0.031	0.036
8	0.010	0.012	0.011	0.011	0.009	0.010	0.019	-	0.035	0.033
9	0.001	0.003	0.002	0.002	0.001	0.001	0.009	0.009	-	0.009
10	0.002	0.003	0.002	0.003	0.001	0.001	0.009	0.007	0.001	-

very low values of genetic distance, as accuracy at this scale can be low.

Discussion

We studied allozyme variation in *Podarcis sicula* under the assumption that the populations living on some Mediterranean islands (Corsica, Elba, Montecristo, Marettimo, Pantelleria) were the result of recent anthropogenic introduction. We therefore expected to find low levels of genetic variability and genetic differentiation in the samples from these islands. The results of the electrophoretic analysis were congruent with these expectations, as all samples from the Tyrrhenian and Sicilian islands were characterized by very low proportions of polymorphic loci and heterozygosity, and were genetically nearly identical to the native populations from the Italian peninsula and Sicily. It must be noticed that the values of genetic distance found between native

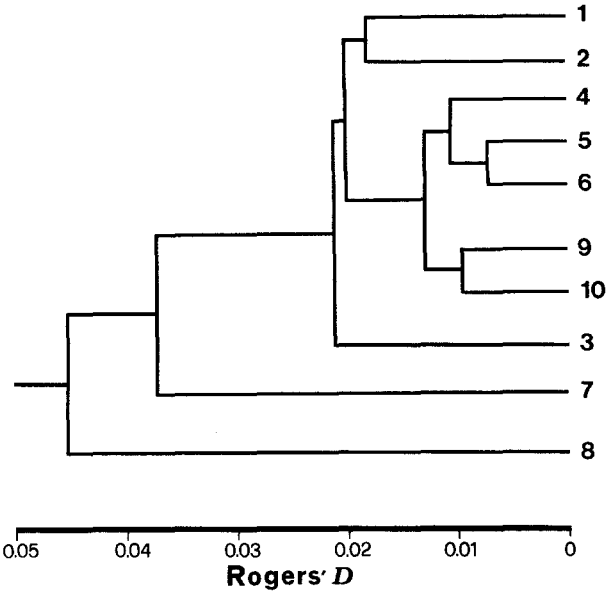

Figure 2. Phenogram generated by UPGMA cluster analysis based on Rogers' genetic distances among populations of *Podarcis sicula*. Cophenetic correlation = 0.927. 1, Firenze; 2, Monte Argentario; 3, Napoli; 4, Caporalino (Corsica); 5, Elba; 6, Montecristo; 7, Milazzo; 8, Selinunte; 9, Marettimo; 10, Pantelleria.

and introduced populations were very similar to, and in some case lower than, those detected within the genus *Podarcis* among local populations of the same species[34,35].

Based on the obtained values of mean number of alleles per locus (*A*) and proportion of polymorphic loci (*P*), viz. two genetic variability parameters which appear to be particularly affected by population bottlenecks[36,37], it can be inferred that the populations of *Podarcis sicula* from Corsica, Elba, and Montecristo were founded by a small number of individuals. The analysis of the allele frequencies would suggest a colonization of these islands by transported specimens coming from central Italy, thus supporting the hypothesis that *P. sicula* is a recent invader. On the same basis and taking into account the results of the cluster analysis, it can be inferred that the populations from Marettimo and Pantelleria were founded by individuals accidentally introduced from peninsular Italy in historical or protohistorical times. The samples from these two islands showed a dramatic reduction in heterozygosity when compared with those from the Italian peninsula and Sicily, suggesting the effect of bottlenecking (e.g. founder effect) on genetic variability. The presumption is that the populations of *P. sicula* inhabiting Pantelleria and Marettimo may have originated from very few individuals.

Within the lacertid lizards, levels of genetic variation are known to be particularly low in relict island populations, and in populations living on small islands, i.e. tiny fringing islands ($\simeq$0.01 square km) that are separated by a short linear distance and shallow channel depth from the mother island[10]. However, the investigated populations from the Tyrrhenian and Sicilian islands cannot be considered relict populations, as they are genetically nearly identical to the native populations from peninsular Italy. Moreover, these islands are large (e.g. Pantelleria: 86 km^2) or very large (e.g. Corsica: 8722 km^2), and are separated from the mainland (Italian peninsula) or from the mother island (Sicily) by a deep and wide sea channel. On the basis of these considerations, and taking into account the great similarity between our results and those from an analogous study carried out on *Anolis* lizards (Reptilia: Iguanidae) from the Lesser Antillean islands[7], we can conclude that the severe reduction in genetic variability and the very low levels of genetic differentiation pointed out in the samples from Corsica, Elba, Montecristo, Pantelleria and Marettimo are consistent with the hypothesis of a recent arrival of *P. sicula* on these islands, and provide additional evidence of loss of genetic variability in insular populations having originated from episodes of human transportation.

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