



Low genetic variation in a critically endangered Mediterranean lizard: conservation concerns for *Podarcis raffonei* (Reptilia, Lacertidae)

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ABSTRACT

The conservation status of *Podarcis raffonei*, native to the Aeolian Islands (north east of Sicily, Tyrrhenian Sea), is assessed by studying its genetic structure and evidencing factors affecting the survival of the species. Genetic variation in the species is quite low and genetic subdivision is high, as compared to other lacertid lizards and to vertebrates in general. Very little gene flow was detected, implying little or no contact between populations. The four remaining populations of *P. raffonei* are under severe threat from the effects of habitat alteration, interspecific competition, collecting and trade, very low population density and inbreeding, population fragmentation, and apparent loss of genetic variation. Possible management and conservation measures are suggested.

KEY WORDS: *Podarcis raffonei* - Lacertidae - Allozyme electrophoresis - Population heterogeneity - Conservation - Aeolian Islands.

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INTRODUCTION

The biota of small islands are known to be more susceptible to change and extinction than those of continental areas (MacArthur & Wilson, 1967; Reid & Miller, 1989). Anthropogenic threats, such as habitat destruction, over-collecting, introduction of non-native organisms and competitive exclusion, may have greater and unpredictable effects on island ecosystems (Riesenberg & Svensen, 1996). For these reasons, island species have a much higher risk of extinction than mainland ones (Reid & Miller, 1989). Therefore, all data available on the genetic structure and conservation of these species are of particular importance.

The conservation status of the Aeolian wall lizard, *Podarcis raffonei* (Mertens, 1952), has recently received attention because it is likely threatened with extinction (Capula *et al.*, 2002). *P. raffonei* is an endangered lacertid lizard, endemic to the Aeolian Islands (north-east of Sicily, Tyrrhenian Sea) (Fig. 1). The Aeolian Islands constitute a case of volcanism produced by rifting. This archipelago is made up of seven large islands (≥ 338 ha) and several islets (each < 29 ha) and is separated from the closest coast of Sicily by a distance of 22 km (± 500 m) and by a sea channel 500-600 m deep. *Podarcis raffonei* is genetically and morphologically related to *P. wagleriana* Gistel (1868) - a lizard endemic to Sicily, the Egadi Islands and the Stagnone Islands - and its full specific status has only recently been recognised (Capula, 1994a). The Aeolian Islands are also inhabited by the Italian wall lizard, *P. sicula* (Rafinesque, 1810). The Italian wall lizard occurs on all large islands as well as on some islets of the Aeolian Archipelago (Capula, 1992, 1993, 1994a; Corti *et al.*, 1997; Corti & Lo Cascio, 1999), while *P. raffonei* is currently confined to one large island (Vulcano, 2120 ha), where it occurs sympatrically with *P. sicula*, and to three small islands (Scoglio Faraglione, 0.5 ha, 0.3 km west of Salina; La Canna, 1 ha, 1.5 km west of Filicudi; Strombolicchio, 0.19 ha, 1.6 km north-east of Stromboli), where it is the only extant



Fig. 1 - *Podarcis raffonei antonnoi* from Vulcano Island. (Photo: M. Capula).

lacertid lizard (Capula *et al.*, 2002) (Fig. 2). *Podarcis raffonei* was categorised as critically endangered by Capula *et al.* (2002), as it faces an extremely high risk of extinction in the wild in the immediate future under the IUCN criteria B1+2 (IUCN, 1994).

Considering the extremely low density of *P. raffonei* on the smallest islets of the Aeolian Archipelago (Scoglio Faraglione, La Canna, Strombolicchio, each with an area <2 ha) and its complete absence from the large islands (with the exception of Vulcano) (Capula *et al.*, 2002), the future introduction of lizards is possible, either to increase population size or to prevent the smallest populations from accidental extinction. It is therefore important for conservation purposes to have precise information on the genetic makeup of the Aeolian wall lizard, so that biologists and conservation-oriented organisations can select appropriate sources when planning introduction or captive breeding programmes.

In this paper, based primarily on allozyme data, the status of *P. raffonei* is assessed by studying its genetic structure, and appropriate management recommendations are provided to ensure conservation of this critically endangered lacertid lizard and its genetic pattern in the Aeolian Islands.

MATERIALS AND METHODS

Sampling

Samples of *P. raffonei* were obtained from four islands of the Aeolian Archipelago. For interspecific comparison, one sample of *P. sicula* from Vulcano and one sample of *P. wagleriana* from a locality in Sicily were also studied. The geographic origin and number of specimens analysed per population are indicated in Table I.

To avoid killing animals or injurious biopsy, approximately 1 cm of the tail of each lizard was taken off following the suggestion made by Mayer and Tiedemann (1985). The tail fragment was kept in Eppendorf reaction tubes (2mL), and stored below -70 °C until electrophoretic analysis was performed. After collecting the

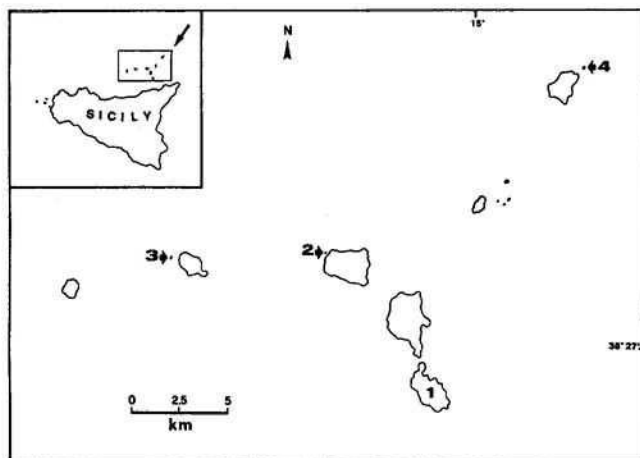


Fig. 2 - Distribution of *Podarcis raffonei* on the Aeolian Islands. 1, Vulcano; 2, Scoglio Faraglione, near Salina; 3, La Canna, near Filicudi; 4, Strombolicchio, near Stromboli. Insert shows location of the Aeolian Islands.

TABLE I - Geographic and collecting data for populations used in the present study.

Species	Locality	n
<i>Podarcis raffonei antoninói</i>	Vulcano (Aeolian Islands)	12
<i>Podarcis raffonei alvearioi</i>	Scoglio Faraglione (Aeolian Islands)	5
<i>Podarcis raffonei cucchiarai</i>	La Canna (Aeolian Islands)	4
<i>Podarcis raffonei raffonei</i>	Strombolicchio (Aeolian Islands)	10
<i>Podarcis sicula sicula</i>	Vulcano (Aeolian Islands)	12
<i>Podarcis wagleriana wagleriana</i>	Palermo (Sicily)	10

piece of tail, lizards were sexed, photographed and then released to the site where they were collected.

Electrophoresis

Standard horizontal starch gel electrophoresis was performed on the tail muscle tissue; parts of this tissue were crushed in 0.1 ml of distilled water. Gene products for the following 23 presumptive enzyme loci were analysed: glycerol-3-phosphate dehydrogenase (E.C. 1.1.1.8, *aGpd*), lactate dehydrogenase (E.C. 1.1.1.27, *Ldb-1*, *Ldb-2*), malate dehydrogenase (E.C. 1.1.1.37, *Mdb-1*, *Mdb-2*), malic enzyme (E.C. 1.1.1.40, *Me-1*, *Me-2*), isocitrate dehydrogenase (E.C. 1.1.1.42, *Idb-1*, *Idb-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*), purine nucleoside phosphorylase (E.C. 2.4.2.1, *Np*), 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*), adenosine deaminase (E.C. 3.5.4.4, *Ada*), carbonate dehydratase (E.C. 4.2.1.1, *Ca-2*), 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*). In addition, three unidentified non-enzymatic proteins, i.e. *Gp-1*, *Gp-2*, *Gp-4* were studied. The buffer systems used, electrophoretic procedures, staining techniques, and loci and allele designations were those described by Capula (1994a).

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. raffonei* populations. Genotypic proportions expected on the basis of the Hardy-Weinberg equilibrium were calculated using 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 & 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 & 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 0 (complete panmixia) to 1 (breeding units fixed for alternative alleles). F_{IS} gives the average inbreeding coefficient of an individual within a breeding unit, and F_{IT} gives the average inbreeding coefficient relative to the total population. F_{IS} and F_{IT} values can be either positive (heterozygote deficiency) or negative (heterozygote excess). Statistical significance of Wright's standardised variance in allele frequencies (F_{ST}) was tested by the χ^2 test following Workman & Niswander (1970). The genetic relationships among the populations studied were evaluated using Nei's (1972) standard genetic identity (I) and standard genetic distance (D). All genetic variability, F -statistics, and genetic distance measures were calculated using the computer program BIOSYS-1 (Swofford & Selander, 1989).

Genotype data at 26 electrophoretic loci were analysed for rates of gene flow (Nm) in *P. raffonei*. Quantitative estimates of Nm were derived using the Wright indirect method (1978). In this method, Nm is related to F_{ST} by the formula:

$$F_{ST} = 1/(1 + 4Nm)$$

where N is the local population size and m the average rate of immigration. Nm ranges from 0 (absence of gene flow) to infinity, and it is considered low (insufficient to prevent populations from diverging genetically) if it is less than 0.25.

RESULTS

Twenty-two of the 26 presumptive gene loci scored (85 per cent) were found to be monomorphic and fixed for the same allele in all the samples of *P. raffonei* (αGpd , $Ldb-1$, $Ldb-2$, $Mdb-1$, $Mdb-2$, $Me-1$, $Me-2$, $Idb-1$, $Idb-2$, $Gapd$, $Sod-1$, Np , $Got-1$, $Got-2$, Ck , Ak , Ada , Gpi , $Pgm-1$, $Gp-1$, $Gp-2$, $Gp-4$). Four loci only (15 per cent) were found to be polymorphic (βPgd , $Ca-2$, Mpi , $Pgm-2$). The locus $Ca-2$ was strongly polymorphic, while the βPgd and Mpi loci were weakly polymorphic. At the $Pgm-2$ locus, where two electrophoretic alleles occur ($Pgm-2^{100}$ and $Pgm-2^{105}$), a particular situation was found. The $Pgm-2^{100}$ allele was fixed in the samples from Vulcano, La Canna and Scoglio Faraglione, whereas the sample from Strombolicchio showed fixation of the unique allele $Pgm-2^{105}$. The lizards from Strombolicchio were characterised by two unique electrophoretic alleles (i.e. alleles found in only one population) also at the $Ca-2$ ($Ca-2^{110}$) and Mpi (Mpi^{105}) loci. Both these alleles were detected at relatively low frequencies.

The results of the contingency χ^2 analysis are given in Table II. The analysis reveals that one ($Pgm-2$) out of four variable loci exhibits statistically significant heterogeneity in the allele frequencies. This result, in accordance with the allele frequencies data indicating fixation of alternative alleles at the $Pgm-2$ locus, shows that there is a certain degree of substructuring among populations of *P. raffonei*.

The measures of genetic variability are given in Table III. All the analysed samples of *P. raffonei* showed noticeably low values of polymorphism and heterozygosity. The overall mean number of alleles per locus was

TABLE II - χ^2 values resulting from contingency χ^2 analysis at the polymorphic loci among populations of *Podarcis raffonei*. d.f. = degrees of freedom; NS, nonsignificant.

Locus	No. of alleles	χ^2	d.f.	P
βPgd	2	3.088	3	NS
$Ca-2$	2	2.057	3	NS
Mpi	2	2.708	3	NS
$Pgm-2$	2	68.000	3	<0.001
Total		75.853	12	<0.001

1.05, ranging from 1.0 to 1.1. The proportion of polymorphic loci (P) ranged from 0.0 (La Canna) to 7.7 (Vulcano, Strombolicchio), averaging 4.8. The observed heterozygosity (H_o) showed a similar trend, ranging from 0.000 (La Canna) to 0.018 (Vulcano) and averaging 0.011. For two populations (Vulcano and Strombolicchio), heterozygosity was slightly higher than that expected under the Hardy-Weinberg equilibrium (Table III). A positive correlation between the proportion of polymorphic loci and heterozygosity was found in all samples (Spearman rank correlation, $r = 0.950$, $P < 0.05$), indicating that in *P. raffonei* P and H_o are closely related.

Table IV provides estimates of F_{IS} , F_{IT} and F_{ST} for all variable loci among *P. raffonei* samples. The four polymorphic loci evaluated for all populations yielded negative F_{IS} values, and mean F_{IS} was also negative (-0.152). These data point to a slight heterozygote excess, as also indicated by the genetic variability data (Table III). The weighted mean value for F_{IT} was positive (0.556). F_{ST} values ranged from 0.042 (Mpi) to 1.000 ($Pgm-2$). The high value found for $Pgm-2$ indicates that this is the only locus in which there is allele fixation. The weighted mean value of F_{ST} was 0.614, indicating that 61 per cent of genetic variation in *P. raffonei* is attributable to differentiation among populations.

TABLE III - Genetic variability parameters in *Podarcis raffonei* populations. A, mean number of alleles per locus; P, mean proportion of polymorphic loci; H_o , observed mean heterozygosity; H_e , expected mean heterozygosity.

Population	A	P	H_o	SE	H_e	SE
Vulcano	1.1	7.7	0.018	0.014	0.016	0.012
Scoglio Faraglione	1.0	3.8	0.008	0.008	0.008	0.008
La Canna	1.0	0.0	0.000	0.000	0.000	0.000
Strombolicchio	1.1	7.7	0.017	0.013	0.016	0.012

TABLE IV - Summary of F -statistics at four variable loci for *Podarcis raffonei* populations.

Locus	F_{IS}	F_{IT}	F_{ST}
<i>6Pgd</i>	-0.077	-0.018	0.055
<i>Ca-2</i>	-0.178	-0.121	0.048
<i>Mpi</i>	-0.059	-0.014	0.042
<i>Pgm-2</i>	—	1.000	1.000
Mean	-0.152	0.556	0.614

The values of standard genetic identity and genetic distance for each pairwise comparison are given in Table V. Nei's standard genetic distance (D) between the *P. raffonei* populations ranged from 0 to 0.041, averaging 0.020. Despite the large geographic distance and the wide sea channel separating the islands of Vulcano, Scoglio Faraglione and La Canna, the values of genetic distance between the samples from these islands were quite low (average $D = 0.0003$). On the other hand, the comparison between the Strombolicchio sample and the other Aeolian samples (Vulcano, Scoglio Faraglione, La Canna) gave the highest distances (average $D = 0.040$; see Table V). This is because the Strombolicchio population is characterised by the fixation of a unique allele at the *Pgm-2* locus and by the occurrence of two unique alleles at the *Ca-2* and *Mpi* loci respectively.

As to the interspecific genetic distances, the comparison between *P. raffonei* and *P. sicula* gave an average Nei's genetic distance ($D = 0.319$) similar to that found between the latter species and *P. wagleriana* ($D = 0.405$). On the other hand, *P. wagleriana* was genetically more closely related to *P. raffonei* ($D = 0.146$), thus confirming the apparent phylogenetic affinity between the two species suggested by Capula (1994a,b).

Estimates of Nm based on Wright's method are low. The Nm value for the total sample of *P. raffonei* is 0.157 (i.e. <0.25). This implies that gene flow between the various populations is not enough to prevent them from diverging genetically.

DISCUSSION

This study indicates that the geographically fragmented populations of *Podarcis raffonei* are characterised by very low levels of genetic variability and by a genetic structure more subdivided than that of other *Podarcis* species studied to date. The Aeolian wall lizard shows values of polymorphism and heterozygosity noticeably lower than (i) those detected in the phylogenetically related *P. wagleriana* from Sicily ($P = 15$; $H_o = 0.037$; Capula, 1994a), (ii) the average ones calculated by Capula (1990, Unpublished PhD. Thesis, Univ. of Bologna) for nine species of the genus *Podarcis* ($P = 13$; $H_o = 0.053$), and (iii) the average ones calculated by Nevo (1978) for 17 species of reptiles ($P = 22$; $H_o = 0.047$). The severe reduction in genetic variability pointed out in *P. raffonei* was also evidenced by molecular analyses (Oliverio *et al.*, 1998). Different hypotheses can be proposed to explain the observed pattern of genetic variation. The first is that loss of alleles and erosion of genetic variability in the populations inhabiting the smallest islands might be the result of genetic drift phenomena, such as population bottlenecks (e.g. founder effect: the original colonisers carry only a subsample of the genetic variability of the parental population; Selander, 1976). The second, not mutually exclusive, hypothesis is that low genetic variation within populations could be also attributed to directional selection, as pointed out by Gorman *et al.* (1975) in insular populations of the related Mediterranean species *P. melisellensis* and *P. sicula*. The third hypothesis is that low genetic variation of the

TABLE V - Values of Nei's (1972) standard genetic identity (above the diagonal) and standard genetic distance (below the diagonal) among populations of *Podarcis raffonei* (P.r.; Vu, Vulcano, SF, Scoglio Faraglione, LC, La Canna, St, Strombolicchio), *P. sicula* (P.s.; Vu, Vulcano), and *P. wagleriana* (P.w.; Pa, Palermo).

Population	P.r. (Vu)	P.r. (SF)	P.r. (LC)	P.r. (St)	P.s. (Vu)	P.w. (Pa)
P.r. (Vu)	—	1.000	0.999	0.961	0.735	0.873
P.r. (SF)	0.000	—	1.000	0.961	0.737	0.874
P.r. (LC)	0.001	0.000	—	0.960	0.738	0.875
P.r. (St)	0.040	0.040	0.041	—	0.696	0.836
P.s. (Vu)	0.308	0.305	0.303	0.362	—	0.667
P.w. (Pa)	0.136	0.134	0.134	0.179	0.405	—

studied populations could be partially caused by their having had low variation before geographic fragmentation (Frankham, 1997). All these hypotheses seem to be consistent when considering that: (i) three out of four extant *P. raffonei* populations occur on tiny fringing islands, each characterised by a very limited area (< 2 ha), that are separated by a short linear distance and shallow channel depth from the mother island; (ii) the populations occurring on Vulcano Island - which is the only large Aeolian island inhabited by the species - are extremely fragmented and are characterised by a very small number of individuals (Capula, 1994a).

The levels of genetic subdivision observed in *P. raffonei* exceed values known for other lizard species (McKinney *et al.*, 1972; Sites & Greenbaum, 1983; Sarre *et al.*, 1990). The estimated standardised variance in gene frequency (F_{ST}) is highly significant, with a value (0.610) much higher than those calculated by Capula (1994a, 1996) for the related insular lacertid lizards *P. wagleriana* ($F_{ST} = 0.153$) and *P. tiliguerta* ($F_{ST} = 0.460$), and very high for vertebrates in general (Gorman *et al.*, 1975; Wright, 1978; Raghianti & Wake, 1986). Allele frequency data, F -statistics and genetic distance data show that, at the scale of the study, genetic variation in *P. raffonei* is distributed to two population groups. The first group includes the populations from the southern (Vulcano) and western (La Canna, Scoglio Faraglione) Aeolian Islands, the second includes the population from Strombolicchio, off the north-eastern coast of Stromboli, i.e. the northernmost island of the Aeolian Archipelago (Figure 1). The rates of gene flow among the populations are noticeably low ($Nm = 0.157$), indicating highly significant independence of local demes. This situation corresponds well to the case of the island model of population differentiation (Wright, 1940, 1943), in which strong isolation and large genetic differences among populations are involved.

Investigations carried out by Capula *et al.* (2002) on the Aeolian islands during 1989-99 gathered evidence that strongly indicates that the endemic *P. raffonei* is close to extinction. According to these authors, each of the three tiny islands inhabited only by *P. raffonei* (Scoglio Faraglione, La Canna, Strombolicchio) support a small number of lizards (estimated to be $20 \leq N \leq 60$). On the other hand, on Vulcano, the species occurs in two localities only, and it has almost reached extinction probably because of competitive exclusion by the very abundant lizard *P. sicula* (Capula, 1993; Capula *et al.*, 2002). Direct evidence for the decline of the Aeolian wall lizard is scarce because there are no previous data (i.e. prior to 2002) on the original distribution and demography of the species, but the decline is indicated indirectly by several factors. According to Capula *et al.* (2002), four main factors are contributing to the species' extinction: 1) habitat alteration (fire, over-grazing, agricultural practices, touristic activities); 2) competitive exclusion by the lacertid lizard *P. sicula*, introduced by humans; 3) collection and trade; 4) very low population

density and inbreeding. Other threats, pointed out in the present paper, are the very reduced gene flow between populations, due to high geographic fragmentation and isolation, and the apparent decline in genetic variability. The amount of genetic variation is believed to be positively correlated to fitness (Lande, 1988; Lynch, 1996), and so the small populations of *P. raffonei* are genetically highly vulnerable to environmental change and human impact. All of the above factors affect the survival of the species and underline the urgent need for implementation of conservation measures. Such measures need to take into account that populations on different islands are genetically different and that as much as possible genetic variation present in the species should be preserved. Moreover, it must be stressed that without a long-term conservation programme, utilising both *in situ* and *ex situ* management, extinction of wild populations might occur in the near future. Therefore we propose several conservation recommendations, which are also of relevance to other Mediterranean islands with threatened lizard populations (e.g. *P. atrata* from the Columbretes Islands). In order to maintain the existing wild populations, collection and trade for commercial purposes should be prohibited by full legal protection, and natural reserves should be created on all islands inhabited by the species. On the same islands, prevention of intentional or accidental introductions of predators and competitor species is required, and habitat conservation measures must be acted upon by preventing any kind of habitat stress. Since existing wild populations are composed of a small number of individuals and thus are vulnerable to stochastic impacts, we recommend the planning of captive breeding programmes as soon as possible. Moreover, we recommend that (i) new populations be established in safe locations within natural and semi-natural habitats (this would imply re-introduction and conservation introduction projects, *sensu* IUCN, 1998), and (ii) documented gene banks be established adjacent to protected areas by creating effective *ex situ* populations.

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