Population heterogeneity and conservation of the Aeolian wall lizard, *Podarcis raffonei*

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The conservation status of Podarcis raffonei*, native to the Aeolian Islands (north-east of Sicily, Tyrrhenian Sea), is assessed analyzing 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. 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.*

Keywords: *Podarcis raffonei*, Lacertidae, population heterogeneity, conservation, Aeolian Islands.

Introduction

Podarcis raffonei (Mertens 1952) is a lacertid lizard endemic to the Aeolian Islands (north-east of Sicily, Thyrrhenian Sea) (Fig. 1). The species is genetically and morphologically related to *P. wagleriana* Gistel 1868, a lizard endemic to Sicily, Egadi Islands and Stagnone Islands (Capula 1994a, b; Capula *et al*. 2001). The Aeolian Islands are also inhabited by *Podarcis sicula* (Rafinesque 1810), but the occurrence of this species in the Archipelago is probably due to introduction by humans in protohistorical times (Capula 1994a, Corti & Lo Cascio 1999, Lo Cascio & Corti 2004). *P. sicula* occurs on all large islands as well as on some islets of the Aeolian Archipelago (Capula 1992, 1993, 1994a; Corti *et al*. 1998; 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 islets (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 lacertid lizard (Capula

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Fig. 1. Adult male *Podarcis raffonei antoninoi* (Vulcano Island).

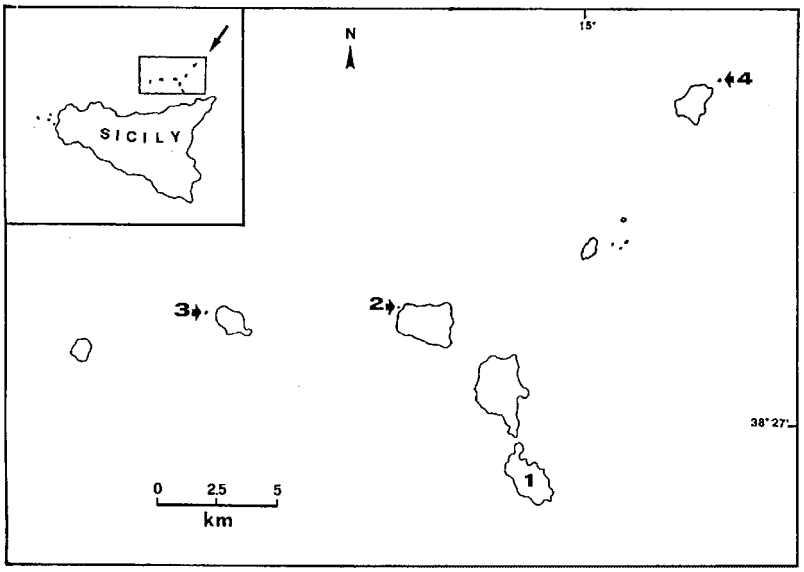

Fig. 2. Distribution of *Podarcis raffonei* in the Aeolian Islands. 1, Vulcano; 2, Scoglio Faraglione, near Salina; 3, La Canna, near Filicudi; 4, Strombolicchio, near Stromboli. Insert shows location of the Aeolian Islands.

et al. 2002) (Fig. 2). The following subspecies are currently recognized: *P. r. raffonei* (Strombolicchio), *P. r. antoninoi* (Vulcano), *P. r. alvearioi* (Scoglio Faraglione), *P. r. cucchiarai* (La Canna) (Capula 1994a, b; Turrisi & Vaccaro 1998).

Because of (i) the extremely low density of *P. raffonei* on the smallest islets of the Aeolian Archipelago (Scoglio Faraglione, La Canna, Strombolicchio, each with an area of 2 < ha) (Capula *et al*. 2002, Capula & Lo Cascio 2006), and (ii) its complete absence from the large islands – with the exception of Vulcano, where the species is at present extremely rare and nearly reaching the extinction – the Aeolian wall lizard was categorised as critically endangered (Capula *et al.* 2002).

In this paper, the status of *P. raffonei* is assessed by reviewing the existing information on the genetic and morphological structure of the species, and by providing management recommendations to ensure its conservation in the Aeolian Islands.

Materials and Methods

Population heterogeneity

The genetic structure of *P. raffonei* populations occurring on Vulcano Island and on Scoglio Faraglione, La Canna and Strombolicchio islets was analyzed and discussed using allozyme variation data at 26 gene loci (α*Gpd*, *Ldh-1, Ldh-2*, *Mdh-1, Mdh-2*, *Me-1, Me-2*, *Idh-1, Idh-2, 6Pgd*, *Gapd*, *Sod-1*, *Np*, *Got-1, Got-2*, *Ck*, *Ak*, *Ada*, *Ca*, *Mpi*, *Gpi*, *Pgm-1, Pgm-2, Gp-1, Gp-2, Gp-4*) provided by Capula (1994a, 2004). 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). 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 by the computer program BIOSYS-1 (Swofford & Selander 1989).

Conservation status

Information concerning the conservation status and the factors affecting the survival of *P. raffonei* is based on field investigations carried out on all large islands of the Aeolian Archipelago (Lipari, Salina, Vulcano, Stromboli, Filicudi, Alicudi, Panarea) and on six islets (Basiluzzo, Bottaro, Lisca Bianca, La Canna, Scoglio Faraglione,

Strombolicchio) during a 16-year research period (1989-2004). All observations were carried out during springtime (April, May) and at the end of summer (September). To describe the main causes affecting the survival of *P. raffonei*, data gathered from the literature concerning the species (Capula 1993, 1994a, 2004; Capula & Luiselli 1997; Capula *et al*. 2002; Corti & Lo Cascio 1999, 2002; Lo Cascio & Pasta 2004; Luiselli *et al*. 2004; Capula & Lo Cascio 2006) were also analyzed and discussed.

Results and Discussion

Population heterogeneity

The small and geographically fragmented populations of *P. raffonei* are characterized by very low levels of genetic variability and by a genetic structure more subdivided than that of other *Podarcis* species studied to date. According to Capula (2004) 22 of the 26 presumptive gene loci scored (85%) were found to be monomorphic and fixed for the same allele in all the samples of *P. raffonei*, and four loci only (15 %) were found to be polymorphic (*6Pgd, Ca, Mpi, Pgm-2*).

All the analysed samples of *P. raffonei* show noticeably low values of polymorphism and heterozygosity (see Table 1). The overall mean number of alleles per locus was 1.05, and the proportion of polymorphic loci (*P*) averaged 4.8 (Capula 2004). The observed heterozygosity (H_o) showed a similar trend, ranging from 0.000 (La Canna) to 0.018 (Vulcano) and averaging 0.011. The Aeolian wall lizard shows values of polymorphism and heterozygosity noticeably lower than those detected in the phylogenetically related *P. wagleriana* from Sicily (*P* = 15; H_o = 0.037; Capula 1994a) and the average ones calculated by Capula (1990) for nine species of the genus *Podarcis* (*P* = 13; H_o = 0.053). This is probably because three out of four extant *P. raffonei* populations occur on tiny fringing islands, each characterized by a very limited area (< 2 ha) and separated by a short linear distance and shallow channel depth from the mother island; on the other hand, the populations occurring on Vulcano Island – which is

Table 1. Genetic variability parameters in *Podarcis raffonei* populations. *A*, mean number of alleles per locus; *P*, mean proportion of polymorphic loci; *Ho*, observed mean heterozygosity; *He*, expected mean heterozygosity; (SE), standard error.

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

the only large Aeolian island inhabited by the species – are extremely fragmented and are characterized by a very small number of individuals (Capula 1994a, Capula *et al*. 2002). The severe reduction in genetic variability pointed out in *P. raffonei* was also evidenced by molecular analyses (Oliverio *et al*. 1998). However, it must be noted that the results by Oliverio *et al.* (1998) concerning the phylogenetic relationships of *P. raffonei* are in disagreement with those pointed out by allozyme (Capula 1994a, b), mtDNA (Harris & Arnold 1999), and morphometric analyses (Capula *et al*. 2001). According to Capula (1994a, b), Harris & Arnold (1999) and Capula *et al*. (2001) *P. raffonei* is phylogenetically related to *P. wagleriana*, while the results by Oliverio *et al.* (1998) would indicate a strict relationship between *P. raffonei* and *P. muralis* (Laurenti 1768). However, on a subsequent paper Oliverio *et al.* (2000) proposed a very different phylogenetic scenario, biogeographically not congruent neither with that reported by Oliverio *et al*. (1998) nor with that by Capula (1994a, b), Harris & Arnold (1999) and Capula *et al.* (2001), suggesting a close affinity between *P. raffonei* and *P. tiliguerta* (Gmelin 1789).

The values of standard genetic identity and genetic distance for each pairwise comparison are given in Table 2. 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 gave the highest distances (average $D = 0.040$). The individuals of this population are characterized by a unique electrophoretic allele (*Pgm-2*^{105}) and are large-sized and morphologically recognizable from those occurring on the other Aeolian Islands (Capula *et al*. 2001, Capula 2004).

According to Capula (2004) the levels of genetic subdivision observed in *P. raffonei* exceded values known for other lizard species (McKinney *et al*. 1972, Sites & Greenbahum 1983, Sarre *et al*. 1990). The estimated standardized variance in gene frequency (F_{ST}) is highly significant (Capula 2004), with a value (0.610) much higher

Table 2. Values of Nei's (1972) standard genetic identity (above the diagonal) and standard genetic distance (below the diagonal) among populations of *Podarcis raffonei* (Vu= Vulcano, SF= Scoglio Faraglione, LC= La Canna, St= Strombolicchio).

Population	Vu	SF	LC	St
Vu	----	1.000	0.999	0.961
SF	0.000	----	1.000	0.961
LC	0.001	0.000	----	0.960
St	0.040	0.040	0.041	----

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, Ragghianti & Wake 1986). These data are congruent with the results of the geometric morphometric analyses (Capula *et al.* 2001). According to the landmark based morphometric analyses of the dermal skulls of twentytwo adult males *Podarcis raffonei* from the four islands of the Aeolian Archipelago (Capula *et al.* 2001), the *P. raffonei* populations are morphologically highly differentiated to each other (see, *e.g.*, Fig. 3). This is probably due to geographic isolation of populations in different islands, and to genetic drift and effects.

Genetic and morphometric data (Capula *et al.* 2001, Capula 2004) indicate that geographic variation in *P. raffonei* is distributed into two population groups. The first group includes the very close populations from the southern (Vulcano) and western (La Canna, Scoglio Faraglione) Aeolian Islands; the second group includes the populations from Strombolicchio, off the north-eastern coast of Stromboli, i.e. the northernmost island of the Aeolian Archipelago. Genetic variability is very low, and genetic and morphometric subdivision is high. According to these results the subspecies *antoninoi* and *cucchiarai* should be synonymized with *alvearioi*. Therefore the following subspecies should be now recognized: *P. r. raffonei* (Strombolicchio), *P. r. alvearioi* (Vulcano, La Canna, Scoglio, Faraglione).

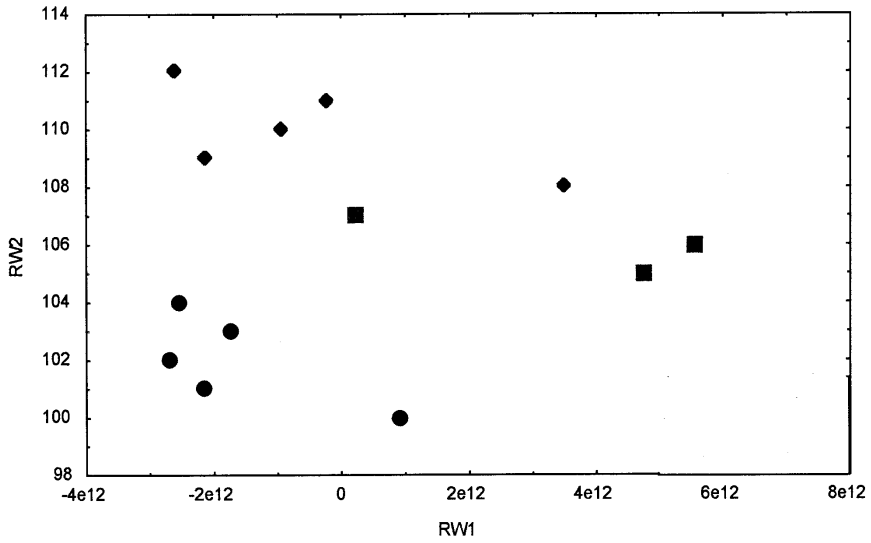

Fig. 3. Ordination of adult males *P. raffonei* (circle: Strombolicchio, n = 5; square: Scoglio Faraglione, n = 3; rhombus: Vulcano, n = 5) along the first two relative warps (RW1, RW2; 31.07% and 25.50% of variation explained respectively) for the cranial dermal skulls (see Capula *et al.* 2001 for more information).

Conservation status

Investigations carried out by Capula *et al*. (2002) and Capula (2004) in the Aeolian Islands gathered evidence strongly indicating that the endemic *P. raffonei* is close to extinction. According to these authors, each of the three tiny islands inhabited only by *P. raffonei* supports a very small number of lizards (Strombolicchio and Scoglio Faraglione: 200-400 individuals; La Canna: 20-30 individuals; Capula & Lo Cascio 2006). On the other hand, on Vulcano the species occurs in two localities only, and it is nearly reaching extinction probably because of competitive exclusion by the very abundant lizard *P. sicula* (Capula 1993, Capula *et al*. 2002, Capula & Lo Cascio 2006, Capula unpublished personal observations). Direct evidence for the decline of the Aeolian wall lizard is scarce because there are no previous data (i.e. prior to 1994) on the original distribution and demography of the species, but the decline is indicated indirectly by several factors. According to Capula *et al.* (2002) and Capula (2004), six main pressures on the species can be listed as contributory factors to extinction: (1) habitat alteration (fire, over-grazing, agricultural practices, touristic activities); (2) competitive exclusion by the lacertid lizard *P. sicula*, introduced by humans in historical times; (3) collecting and trade; (4) very low population density and inbreeding; (5) very reduced gene flow between populations, due to high geographic fragmentation and isolation; (6) 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 changes and human impacts. All of the above factors probably affect the survival of this insular species and underline the urgent need for implementation of conservation measures (Reid & Miller 1989).

Podarcis raffonei and its habitat are still not protected by the Italian law or by any international convention concerning conservation and protection of vertebrate fauna. The species requires urgent protection as it can be considered the most threatened lizard in Italy and possibly in the whole Europe (Capula *et al*. 2002, Corti & Lo Cascio 2002, Lo Cascio & Pasta 2004). *Podarcis raffonei* may yet be saved from extinction if the following recommendations are acted upon:

- The last wild populations should be officially protected as soon as possible by creating natural reserves.
- Collection and trade for commercial purposes should be forbidden by full legal protection to reduce the risk of depletion of wild populations.
- Habitat conservation measures should be instigated by monitoring and managing areas where the natural habitat of the species still persists.
- Control of the presence of alien fauna and prevention of intentional or accidental introductions of predators (*e.g. Hierophis viridiflavus*) and competitor species (*e.g. Podarcis sicula*) is required.

- Continuity of scientific studies on relict populations should be promoted in order to document annual fluctuations in population parameters.
- It is necessary to avoid habitat stress on the islands inhabited by the species and to provide for habitat restoration.
- It is necessary to prevent gene flow between islands.
- Since the existing wild populations are composed by a small number of individuals and thus are vulnerable to stochastics impacts, it is urgent to plan captive breeding programmes.
- Documented gene banks should be established adjacent to protected areas by creating effective *ex situ* populations (IUCN 1998).
- *Podarcis raffonei* should be included in the IUCN Red List of Threatened Species as "Critically Endangered" (see Capula *et al*. 2002; Capula & Lo Cascio 2006).

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