Genetic evidence of natural hybridization between *Podarcis sicula* and *Podarcis tiliguerta* (Reptilia: Lacertidae)

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Abstract. An allozyme survey revealed instances of natural hybridization between *Podarcis sicula* and *P. tiliguerta* from a locality of southern Sardinia. Pure specimens of both species together with some F_1 hybrids were found, but no evidence of backcrossing was detected. The hybrid specimens appeared morphologically intermediate between the parent species. The absence of backcrossed individuals indicate that free interbreeding between the two species is unlikely. Hybrids were found in areas of habitat disturbance only.

Introduction

Little is known on the natural hybridization in lacertid lizards. Some evidence of hybridization has been reported within the genera *Lacerta* and *Podarcis* by Mertens (1950, 1956, 1964, 1968), Arnold (1973), and Mayer and Tiedemann (1985), but in all these cases hybridization was assumed from morphologically intermediate animals or from individuals arising from captive breeding of parent species. The best documented cases of successful natural hybridization are those involving speciation by hybridization pointed out in the so-called *Darevskia saxicola* Complex (Darevsky, 1967). The electrophoretic analysis of bisexual and unisexual species of this Caucasian polymorphic group indicates that the parthenogentic forms arose as a result of hybridization of distinct biological species (see Uzzell and Darevsky, 1975; Moritz et al., 1992).

Within the genus *Podarcis,* only three cases of natural hybridization, documented by allozyme data, are known. The first is a population of *P. melisellensís* from the Adriatic island of Pod Mrčaru (Dalmatia) (Gorman et al., 1975). In this case hybridization and introgression were strongly suspected, as this population is characterized by two alleles electrophoretically identical to alleles found in *P. sicula.* The second case is that of some populations of *P. sicula* and *P. raffonei* from the Aeolian Islands (off NE Sicily) (Capula, 1993). Pure specimens of *P. sicula* and *P. raffonei* together with F_1 hybrids were found by Capula (1993) on Vulcano Island (Aeolian Islands), where the hybrid zone was relatively wide and a high rate of hybridization was detected. Moreover, evidence of eventual past hybridization and introgression between *P. sicula* and *P. raffonei* was documented on Lipari Island (Aeolian Islands), where the local *P. sicula* population seems to have incorporated in its genome some genetic character of a now extinct population of *P. raffonei* (Capula, 1993). The third case is that of the populations of *P. sicula* and *P. wagleriana* from Marettimo Island (Egadi Islands, off W Sicily) (Capula, 1993). On Marettimo, pure specimens of both species together with F_1 hybrids were found, and limited evidence of backcrossing was also detected. On this island the hybrid zone was narrow and located in an area of habitat disturbance (Capula, 1993).

In this paper the first genetic instance of natural hybridization between *P. sicula* and *P. tiliguerta* is reported. This phenomenon was detected studying allozyme variation in a number of populations of *P. sicula* and *P. tiliguerta* from Sardinia, Corsica and their satellite islands and islets (Capula, 1996). *Podarcis sicula* occurs as autochthonous species in peninsular Italy, Sicily, along the Adriatic coast of Dalmatia, and in a number of Tyrrhenian and Adriatic islands (Henle and Klaver, 1986). *Podarcis tiliguerta* inhabits Corsica, Sardinia and many neighbouring small islands (Arnold and Burton, 1978; Lanza, 1986; Gasc et al., 1997). In Sardinia, both *P. tiliguerta* and *P. sicula* occur, but their pattern of distribution on this island is not interdigitating, as the two species very rarely may coexist in the same microhabitat (Arnold, 1987; Capula and Luiselli, 1994; Corti and Lo Cascio, 1999; pers. obs.). According to some authors (Lanza, 1983; Corti and Lo Cascio, 1999), the occurrence of *P. sicula* in Sardina is probably due to historical antrophogenic introduction, as (i) the species is not as widespread on this island as *P. tiliguerta*, and (ii) it does not occur on several Corsican and Sardinian neighbouring islands.

At the morphological level, identification of *P. tiliguerta* and *P. sicula* in the localities where they coexist may be difficult. In fact, these two lacertid lizards are similar in most anatomical features, differing slightly only in colour pattern (*P. tiliguerta* typically has dark markings on its throat, while *P. sicula* has a throat that is white and unspotted, cf. Arnold and Burton, 1978). On the other hand, the detection of electrophoretically diagnostic loci between the two species allows a correct identification of all individuals, either in allopatry or in overlapping areas (Capula, 1990, 1996).

Materials and Methods

Samples were obtained from five localities of Sardinia, between June 1996 and September 1998 (fig. 1). In one out of these localities (Capoterra) *P. sicula* and *P. tiliguerta* were sympatric. In addition, one sample of *P. sicula* from a locality of central Italy (Roma) was also studied to compare allopatric populations of both species. The electrophoretic analysis was undertaken for 80 specimens from all six localities (see table 1).

To avoid killing animals or injurious biopsy, approximately 1 cm of the tail of each lizard was taken off by following the suggestion of Mayer and Tiedemann (1985). After collecting the piece of tail, lizards were sexed, photographed and then released to the site where they were collected.

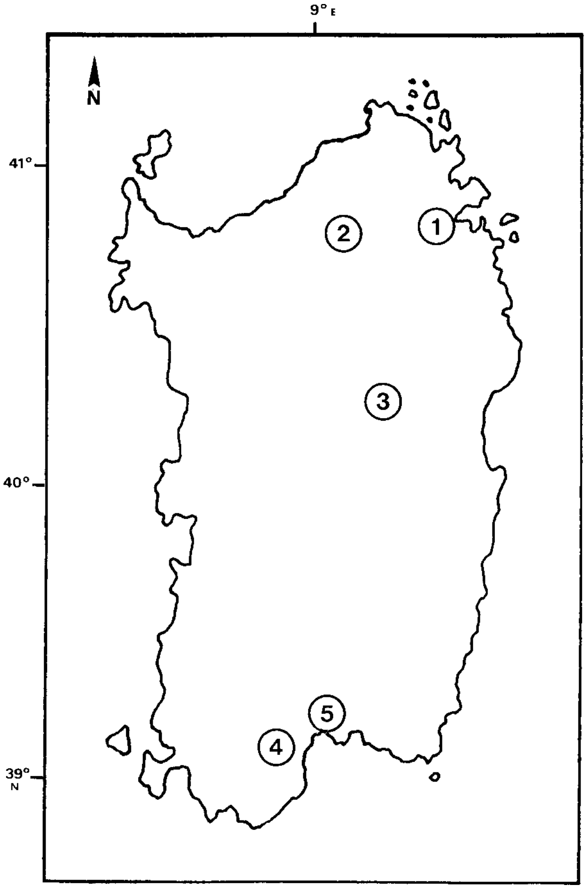

Figure 1. Map of Sardinia showing localities from which samples were examined biochemically. 1) Olbia; 2) Tempio Pausania; 3) Nuoro; 4) Capoterra; 5) Cagliari. The population of *P. sicula* from Roma is outside the range of this map.

Table 1. Collecting localities for *Podarcis tiliguerta* and *P. sicula,* and numbers of parental types (N_p) and hybrids (N_h) identified electrophoretically from each site.

Locality	Species	N_p	N_h
1. Olbia	*P. tiliguerta*	14	0
2. Tempio Pausania	*P. tiliguerta*	19	0
3. Nuoro	*P. tiliguerta*	7	0
4. Capoterra	*P. tiliguerta*	10	3
	P. sicula	10	
5. Cagliari	*P. tiliguerta*	8	0
6. Roma	*P. sicula*	9	0

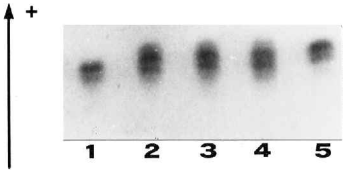

Figure 2. Glutamate-oxaloacetate transaminase-1 (*Got-1*) phenotypes for *Podarcis sicula*, F$_1$ hybrids *P. sicula* × *P. tiliguerta*, and *P. tiliguerta.* (1) *P. sicula* (slow allelic product); (2-4) F$_1$ hybrids *P. sicula* × *P. tiliguerta* (triple banded pattern indicating heterozygous genotype for this dimeric enzyme); (5) *P. tiliguerta* (fast allelic product).

The tail fragment was kept in Eppendorf reaction tubes (2 mL), and stored below -70°C until electrophoretic analysis. 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, *αGpd*), 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, 1996). Only four loci showed different allele frequencies between the two species (diagnostic loci), i.e. *Idh-1, Got-1, Mpi, Gpi.* These loci were found to be diagnostic either between allopatric or sympatric populations of *P. sicula* and *P. tiliguerta* (cf. Capula, 1996). At these loci no common allele was shared between *P. sicula* and *P. tiliguerta,* and hybrids were characterized by heterozygous patterns (see fig. 2).

The amount of genetic divergence between populations was evaluated using Nei's (1972) standard genetic identity (I) and standard genetic distance (D). 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, Nei, 1978). All genetic variability and genetic distance measures were calculated by the computer program BIOSYS-1 (Swofford and Selander, 1989).

Results

Genetically pure *P. sicula* ($n = 9$) and *P. tiliguerta* ($n = 48$) were found in five out six localities studied (see table 1). The samples analyzed always showed fixed differences at the four diagnostic loci. *Podarcis sicula* was homozygous for the following alleles: *Idh-1*^{100}, *Got-1*^{100}, *Mpi*^{100}, *Gpi*^{100}. *Podarcis tiliguerta* was characterized by the following alleles at the same loci: *Idh-1*^{108}, *Got-1*^{104}, *Mpi*^{98}, *Gpi*^{95}. The populations of *P. sicula* and *P. tiliguerta* from these localities were genetically quite distinct, with Nei's average $D = 0.319$ based on the analysis of 20 loci (10 monomorphic: *αGpd*, *Ldh-2*, *Mdh-2*, *Me-2*, *Idh-2, Sod-1, Got-2, Ck, Ak, Pgm-1*; 10 polymorphic: *Ldh-1, Mdh-1, Me-1, Idh-1, 6Pgd, Gapd, Got-1, Mpi, Gpi, Pgm-2*). The values of genetic distance for each pairwise comparison are given in table 2. Within *P. tiliguerta*, Nei's D ranged from 0.007 (between Olbia and Nuoro) to 0.059 (between Nuoro and Cagliari), averaging 0.030. Similar Nei's D values were found comparing the *P. sicula* populations from the Italian peninsula and Sardinia (see table 2). The genetic variability parameters considered here are

Table 2. Values of Nei's standard genetic distance (below the diagonal) and standard genetic identity (above the diagonal) among populations of *Podarcis tiliguerta* (*P.t.*) and *P. sicula* (*P.s.*).

Population		Olbia (*P.t.*)	Tempio Pausania (*P.t.*)	Nuoro (*P.t.*)	Capoterra (*P.t.*)	Cagliari (*P.t.*)	Roma (*P.s.*)	Capoterra (*P.s.*)
Olbia	(*P.t.*)	–	0.989	0.993	0.955	0.949	0.744	0.805
Tempio Pausania	(*P.t.*)	0.011	–	0.988	0.983	0.971	0.714	0.776
Nuoro	(*P.t.*)	0.007	0.012	–	0.957	0.943	0.735	0.794
Capoterra	(*P.t.*)	0.046	0.017	0.044	–	0.980	0.658	0.715
Cagliari	(*P.t.*)	0.052	0.030	0.059	0.020	–	0.643	0.699
Roma	(*P.s.*)	0.295	0.337	0.308	0.418	0.442	–	0.945
Capoterra	(*P.s.*)	0.217	0.254	0.230	0.336	0.357	0.056	–

Table 3. Genetic variability parameters in the studied populations of *P. tiliguerta* (*P.t.*) and *P. sicula* (*P.s.*). 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).

	Olbia (*P.t.*)	Tempio Pausania (*P.t.*)	Nuoro (*P.t.*)	Capoterra (*P.t.*)	Cagliari (*P.t.*)	Roma (*P.s.*)	Capoterra (*P.s.*)
A	1.4	1.5	1.5	1.1	1.1	1.0	1.0
P	0.25	0.35	0.35	0.15	0.10	0.05	0.05
H_o	0.041	0.082	0.100	0.030	0.038	0.020	0.017
H_e	0.079	0.091	0.110	0.043	0.041	0.018	0.017

reported in table 3. The proportion of polymorphic loci (P) in *P. tiliguerta* ranged from 0.10 (Cagliari) to 0.35 (Tempio Pausania and Nuoro), averaging 0.24. The overall mean observed heterozygosity (H_o) was 0.058, and ranged from 0.030 to 0.100. The two populations of *P. sicula* showed noticeably lower values of percent polymorphism (average $P = 0.05$) and heterozygosity (average $H_o = 0.015$) (see table 3).

Natural hybridization was detected at a locality sited ca. 3 km NW of Capoterra (southern Sardinia), where the two species occurred sympatrically. At this locality, genetically pure *P. sicula* and *P. tiliguerta*, together with F$_1$ hybrids were found, and the rate of hybridization was relatively low (frequency of hybrids = 0.130). Table 1 shows the frequencies of parental types and presumptive F$_1$ hybrids. F$_1$ hybrids were characterized by the following heterozygous genotypes at the four diagnostic loci: *Idh-1*$^{100/108}$, *Got-1*$^{100/104}$, *Mpi*$^{98/100}$, *Gpi*$^{95/100}$. No backcrossed individuals were found, thus indicating absence of introgression between the two species. Hybrid specimens appeared morphologically intermediate between the parent species (fig. 3).

Podarcis sicula and *P. tiliguerta* were found to coexist only in degraded fields and secondary maquis habitats. *Podarcis tiliguerta* is widespread at this area, while *P. sicula* seems to be very localized, occurring almost exclusively in very altered habitats. The two

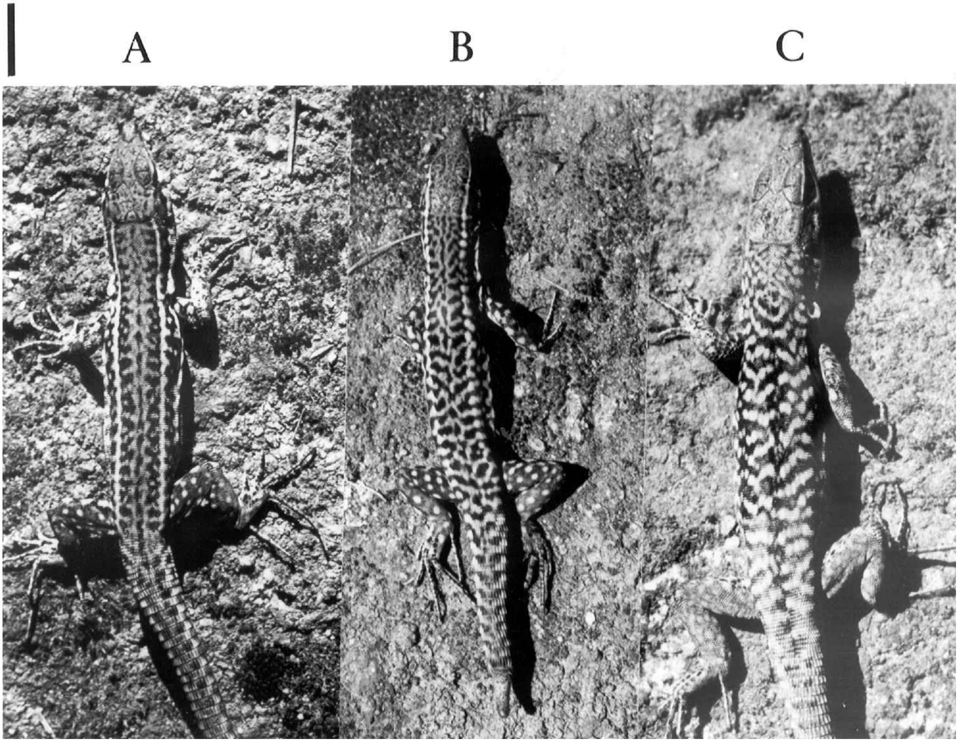

Figure 3. Morphological pattern of *P. tiliguerta* (A), F_1 hybrid *P. sicula* × *P. tiliguerta* (B), *P. sicula* (C) from the locality Capoterra (southern Sardinia). Scale bar = 2 cm.

species seem to meet and hybridize in a very narrow zone only (no more than 1 km in width).

Discussion

Guillaume and Lanza (1982) and Capula (1996) did not find hybrids between *P. sicula* and *P. tiliguerta*. On the other hand, in the present paper evidence of natural hybridization between *P. sicula* and *P. tiliguerta* was observed at a single small area of southern Sardinia. This fact, and the absence of backcrossed individuals, testify to the existence of effective isolating mechanisms and indicate that free interbreeding between the two species is unlikely. Nevertheless, we cannot totally exclude that backcrossed individuals were not detected simply because they were very rare in the field.

The occurrence of natural hybridization between *P. sicula* and *P. tiliguerta* — though observed in only one locality — points to a relative phylogenetic affinity between the two species (see Capula, 1996; Oliverio et al., 1998).

The extension of the hybrid zone between *P. sicula* and *P. tiliguerta* seems to be narrow, as the occurrence of hybrids is restricted to the limited contact area between the two species. However, more data on a larger part of the contact zone are needed in order to allow firm conclusions.

Podarcis sicula is always involved in all the cases of natural hybridization between *Podarcis* species pointed out to date (e.g., *P. sicula* × *P. melisellensis*, Gorman et al., 1975; *P. sicula* × *P. wagleriana*, Capula, 1993; *P. sicula* × *P. raffonei*, Capula, 1993; *P. sicula* × *P. tiliguerta*, this paper). In all these cases, hybrids were observed in central Mediterranean islands, i.e. Pod Mrčaru Island (Dalmatia), Marettimo Island (Egadi Islands, Italy), Vulcano Island (Aeolian Islands, Italy), and Sardinia respectively. It should be stressed that the occurrence of *P. sicula* on the above mentioned islands is probably the result of human transportation followed by acclimatization (Capula, 1992, 1993, 1994; Lanza, 1983; Corti and Lo Cascio, 1999). *Podarcis sicula* appears to be a successful island colonizer (see Nevo et al., 1972; Capula, 1994; Oliverio et al., 2001), and from the above mentioned data it is evident that this lizard, when coming into contact with genetically related species of the genus *Podarcis*, may occasionally hybridize in narrow overlap zones, especially in habitats altered by human activities (Capula, 1993). This seems to be also the case pointed out in our paper, and we can put forward the hypothesis that natural hybridization between *P. sicula* and *P. tiliguerta* in southern Sardinia is the result of a recent overlap of two phylogenetically related species, in which pre-mating isolating mechanisms may have not operated owing to several causes, such as some kind of environmental perturbation. It is well known that cases of natural hybridization are often associated with habitat alteration (Woodruff, 1973; Hillis et al., 1984; Capula, 1993; Bressi et al., 2000). In the case of the *Podarcis* lizard populations from Capoterra, habitat disturbance related to human activity (deforestation, fire, agriculture) may have influenced both the present distribution of the species and the extent of sympatry. This hypothesis requires further investigation, but it is more than a coincidence that in the investigated area natural hybridization is restricted to such environmental circumstances.

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