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Molecular characterization of native (Italy) and introduced (USA) *Podarcis sicula* populations (Reptilia, Lacertidae)

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ABSTRACT

The wall lizard *Podarcis sicula* has been introduced in many locations outside its native range. Partial sequences from the 12S rDNA, that had proved diagnostic for the three main subspecies, were used to test specimens from marginal native and from introduced American populations. The mitochondrial haplotype of a specimen from Apulia, traditionally ascribed to *P. s. campestris*, belongs to the *P. s. sicula* gene-pool. Specimens from a *P. s. sicula* - *P. s. campestris* boundary area (southern Latium), with transitional morphology but *P. s. sicula*-like ecology, show mitochondrial haplotypes clearly of the *campestris* type. The mitochondrial haplotypes of the American specimens (from Kansas and New York), that originated from captive stocks, were included in a *P. s. campestris* clade. A definitive assessment of the status of both native and introduced populations will require the use of nuclear markers.

KEY WORDS: *Podarcis sicula* - Introduced species - Wall lizards - Molecular identification - mtDNA.

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INTRODUCTION

The mtDNA of wall lizards of the genus *Podarcis* has recently been analysed phylogenetically (Oliverio *et al.*, 1998, 2000; Harris & Arnold, 1999). A comparison between the two most widespread Italian species, namely *P. sicula* (Rafinesque-Schmaltz, 1810) and *P. muralis* (Laurenti, 1802), evidenced different degrees of molecular heterogeneity. *Podarcis muralis* is phenotypically extremely variable in both its continental and insular Italian range, which is contrasted by a very low level of molecular divergence. Instead, *P. sicula* is more homogeneous morphologically throughout its range (with several exceptions in small islands), but the existence of at least three subspecies, that traditionally are morphologically based, has been supported by molecular data (Oliverio *et al.*, 1998): *P. s. sicula*, in southern Italy and Sicily, *P. s. campestris* (De Betta, 1857) from northern to central Italy and Corsica, and *P. s. cettii* (Cara, 1872) primarily endemic to Sardinia. The levels of divergence among the tested subspecies (based on specimens from the core of the ranges) were high and seem to demonstrate substantial isolation (especially for the Sardinian *cettii*, which remarkably proved the most primitive). In the smallest islands of the Tyrrhenian and Adriatic seas several insular populations had been separated at the subspecific level (see Lanza, in Amori *et al.*, 1993, for an updated list): their taxonomy is worthy of reconsideration and most of them can probably be synonymized with either *campestris*, *sicula*, or *cettii* (see e.g., Corti *et al.*, 1989, *Abstract* in 1st World Congr. of Herpetol., Canterbury; Capula, 1994).

Podarcis sicula has been introduced in many locations outside its native range. Non-native populations are known in continental Spain, Minorca Is., France, and western Turkey (reviewed in Capula, 1994). In North America, *P. sicula* populations have been established in Philadelphia (PA), Topeka (KS) and on Long Island (NY) (Behler & King, 1979; Conant & Collins, 1991). The PA population (Philadelphia County) was originally reported as *Lacerta mellisellensis* by Kauffeld (1931); Conant (1959) confirmed that the population was then still extant, and referred it to *Lacerta* (*Podarcis*) *sicula campestris*. After extensive searches in 1998 and 1999 of the area where they were originally found, it appears that the PA population is now extinct (Gilmore, Warney, and Burke, pers. obs.).

The south Topeka, Shawnee County (KS), population of *P. sicula* is apparently the result of numerous accidental or deliberate releases of pet animals in the late 1950's (Collins, 1982; Gubanyi, 1999). The source of the releases was Quivera Specialties, a wholesale and retail animal seller at 4010 West 21st Street. Both species were seen in large numbers soon afterwards, indicating that populations grew quickly. *Podarcis sicula* continue to be extremely common in the area around the original release site and have spread to an approximately 2.6

km^2 area around it, although it is not known whether their range is still expanding (Miller, pers. commun.).

The Long Island population of *P. sicula* is the result of a single accidental release of pet animals in 1966, in West Hempstead, Nassau County (NY) (Gossweiler, 1975). By 1972 they had spread at least 0.4 km from the release site, and by 1985 to the neighbouring communities of Garden City South and Franklin Square, occupying an area of approximately 1 km^2 (Garber, 1985). *Podarcis sicula* is currently very common in some urban Long Island habitats, and this population is clearly expanding along the railroad lines that run the length of the island. Their range is no longer continuous; i.e., isolated (apparently secondarily introduced) demes are known in Long Beach, Plandome Manor, and Upper Brookville, as much as 15 km from the original release site. They have also been reported from the Bronx, another highly urbanized area, at least 23 km and one significant river away.

In a recent work (Oliverio *et al.*, 1998), partial DNA sequences of the mitochondrial gene encoding the 12S ribosomal RNA (12S rDNA) proved diagnostic for specimens of the three main subspecies originating from the core of their subspecific ranges. We decided to test this molecular marker on marginal populations. One specimen was from a population (Lecce, Apulia, southern Italy) morphologically referable to the *P. s. campestris* subspecies, at the southern edge of the subspecies range. Three specimens, with transitional morphological characters, were from a boundary area between the nominate and the *campestris* subspecies (Formia, Latium, central Italy). Then, we assayed specimens from the two surviving introduced American populations (KS and NY), to examine the origin of their mitochondrial gene-pool.

MATERIALS AND METHODS

Four sequences from a previous work (Oliverio *et al.*, 1998) were employed as representative of the three main subspecies: *P. s. sicula, P. s. campestris* and *P. s. cettii*. The new Italian samples and those from the introduced American populations were coded by numbering the Ps# and PUSA# acronyms, respectively. *Podarcis pityusensis* (Boscá, 1883) was used as outgroup with a sequence from a previous work (Ppi#1: Oliverio *et al.*, 2000). Collecting data, along with GenBank accession number of the relevant sequences, are reported in Table I.

For DNA extraction from the Italian specimens Pss#5, Psc#1, Psc#3 and Pse#4 see Oliverio *et al.* (1998). For the Balearic Ppi#1 (Oliverio *et al.*, 2000), for all American samples, and for the Italian Ps#6, Ps#7, Ps#8, and Ps#9, the tail-tips were cut in the field and stored in pure ethanol, while drops of blood were absorbed on stripes of sterile 3M Whatmann paper and the specimens released. The material was processed with Proteinase K and standard phenol-chloroform extraction procedures, and DNA was precipitated with isopropanol.

Purified total DNA was used as template for the double-stranded polymerase-chain-reaction (PCR) amplification (see condition in Oliverio *et al.*, 1998). The sequences available after previous works (Oliverio *et al.*, 1998, 2000) included a longer portion of

TABLE I - *Sample acronyms, collecting data, GenBank accession numbers and published source, for sequences used in the present work.*

Code	Locality	GenBank ac. no.	Source
Ppi#1	Maiorca Island, (Baleares, Spain)	AJ250158	Oliverio *et al.*, 2001
Psc#1	Maccarese, near Rome (Latium, Italy)	AJ001474	Oliverio *et al.*, 1998
Psc#3	Rome, Prato Falcone (Latium, Italy)	AJ001475	Oliverio *et al.*, 1998
Pss#5	Sapri (Campania, Italy)	AJ001477	Oliverio *et al.*, 1998
Pse#4	Is Aruttas dunes (W Sardinia, Italy)	AJ001476	Oliverio *et al.*, 1998
Ps#6	Lecce (16.vi.1999, A. Venchi leg.)	AJ278031	This work
Ps#7	Oasi di Gianola (LT), 41°15.98' N - 013°40.74' E (9.vi.1998, M. Bologna, A. Venchi & P. Bombi legg.), female	AJ278032	This work
Ps#8	Maranola, Mt Campone (LT), 41°18.24' N - 013°36.68' E (9.vi.1998, M. Bologna, A. Venchi & P. Bombi legg.), male	AJ278033	This work
Ps#9	Mt Castellone, Terracina, Monte S. Biagio 41°21.78' N - 013°18.00' E (23.vi.1999, M. Bologna & P.Bombi legg.), male	AJ278034	This work
PUSA#435	Carle Place (at the corner of Glen Cove Road and Old Country Roads, 2 5 July 1998) and Oceanside (southern end of Daily Avenue, 4 August 1998), Nassau County, Long Island, New York, USA,	AJ278023	This work
PUSA#436		AJ278030	This work
PUSA#501		AJ278024	This work
PUSA#502		AJ278025	This work
PUSA#593	Topeka (near the corner of 21st Street and Gage Boulevard, 19 September 1998), Shawnee County, Kansas, USA	AJ278022	This work
PUSA#594		AJ278029	This work
PUSA#595		AJ278026	This work
PUSA#596		AJ278027	This work
PUSA#597		AJ278028	This work

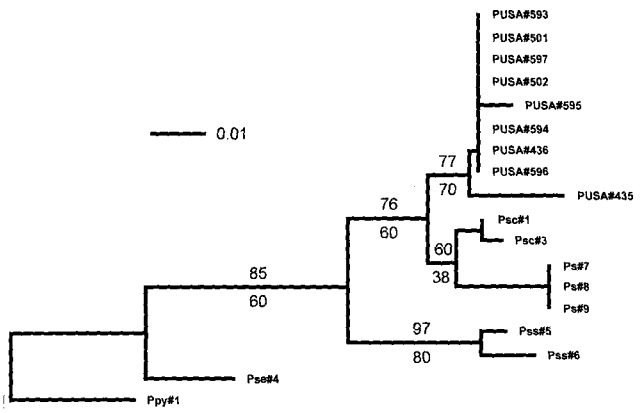

Fig. 1 - Neighbor-joining tree (scale is Tajima-Nei distance): the topology was nearly identical under a branch-and-bound MP analysis. Numbers are the bootstrap support (1000 replicates) of the relevant node in NJ (above branches) and MP (below branches: gaps included) analyses. Note that excluding the gaps in a MP analysis we scored bootstrap values very close to that of the NJ. This topology has the following statistics in MP analyses: gaps included - length = 62, CI = 0.8387, HI = 0.1613, RI = 0.8039; gaps excluded - length = 45, CI = 0.9111, HI = 0.0889, RI = 0.8788.

the 12S gene. In this work, we adopted only the 3' half of that portion, using the primer for the 3' end of the region 5'-AGAACAGGCTCCTCTAGG-3', and a primer designed from a conserved region internal to the gene 5'-ATTTCGTGCCAGCCAC-3'. Sequencing was performed by an ABI-373A automated DNA sequencer using Dye Terminator Ready Reaction Kit (Perkin Elmer).

Nucleotide sequences were aligned by hand, and no ambiguous alignment positions were scored. The divergence (uncorrected 'p' and Tajima-Nei indices) between the sequences was calculated. The aligned sequences were then analysed by the neighbour joining (NJ: Saitou & Nei, 1987) and maximum parsimony (MP: Farris, 1970) methods. Node support in the resulting tree was estimated by 1000 bootstrap replicates; the Ts / Tv ratio was then estimated along the trees. Indels (positions including insertions / deletions, aligned by gaps) were included in the MP analysis, given the very close relationships among the ingroup taxa. Equal weight was given to transitions and transversions. All analyses were performed using the licensed package PAUP 4* (Ver. 4.0b4a, Swofford, 1999).

RESULTS AND DISCUSSION

Alignment of the *P. sicula* sequences with the sequence of Ppi#1 resulted in 313 nucleotide positions. Of these, 19 positions (14, excluding gap positions) contained phylogenetically informative base substitutions within the dataset.

Sequence divergence (Table II) with the outgroup Ppi#1 ranged from 6.6% to 8.1%. Within the *sicula* specimens, it ranged from 0.0% to 4.2%. Within the ingroup, transitions largely outnumbered transversions, as expected when short evolutionary time is involved. The t-ratio (transition/transversion in pairwise comparisons) ranged within the ingroup from 2 to 12. With the outgroup, the t-ratio was 1.4-2.1 (with transversion approaching the number of transitions). The NJ and the MP analyses yielded trees with the same general topology (Fig.1).

Native populations

In the case of the Lecce specimen (Ps#6), the native population was morphologically referred to the *campestris* subspecies, which agrees with a traditional attribution of all Adriatic populations. Its mitochondrial genotype, on the contrary, belongs unequivocally to the *P. s. sicula* genotype. Ps#6 plotted with Pss#5 with high (MP: 80%) to very high (NJ: 97%) bootstrap support. Three specimens were from a boundary area between the nominate and the *campestris* subspecies (Formia, Latium, central Italy), with transitional morphological characters. The populations from this area were attributed to the subspecies *campana*, now synonymyzed with *P. s. sicula*. Their ecology links them to the typical *P. s. sicula* of southern Italy (Marangoni & Bologna,

TABLE II - *Pairwise distances (uncorrected 'p', below diagonal; Tajima-Nei above diagonal) between* Podarcis sicula *mtDNA haplotypes.*

PUSA#593	-	0.0097	0.0000	0.0000	0.0032	0.0000	0.0000	0.0000	0.0000	0.0065	0.0099	0.0266	0.0369	0.0333	0.0163	0.0163	0.0163	0.0732
PUSA#435	0.0096	-	0.0097	0.0097	0.0130	0.0097	0.0097	0.0097	0.0097	0.0135	0.0168	0.0336	0.0442	0.0437	0.0263	0.0263	0.0263	0.0843
PUSA#501	0.0000	0.0096	-	0.0000	0.0032	0.0000	0.0000	0.0000	0.0000	0.0065	0.0099	0.0265	0.0368	0.0331	0.0163	0.0163	0.0163	0.0730
PUSA#502	0.0000	0.0096	0.0000	-	0.0032	0.0000	0.0000	0.0000	0.0000	0.0065	0.0099	0.0265	0.0368	0.0331	0.0163	0.0163	0.0163	0.0730
PUSA#595	0.0032	0.0128	0.0032	0.0032	-	0.0032	0.0032	0.0032	0.0032	0.0098	0.0132	0.0300	0.0403	0.0365	0.0196	0.0196	0.0196	0.0766
PUSA#596	0.0000	0.0096	0.0000	0.0000	0.0032	-	0.0000	0.0000	0.0000	0.0065	0.0099	0.0265	0.0368	0.0331	0.0163	0.0163	0.0163	0.0730
PUSA#597	0.0000	0.0096	0.0000	0.0000	0.0032	0.0000	-	0.0000	0.0000	0.0065	0.0099	0.0265	0.0368	0.0331	0.0163	0.0163	0.0163	0.0730
PUSA#594	0.0000	0.0096	0.0000	0.0000	0.0032	0.0000	0.0000	-	0.0000	0.0065	0.0099	0.0265	0.0368	0.0331	0.0163	0.0163	0.0163	0.0730
PUSA#436	0.0000	0.0096	0.0000	0.0000	0.0032	0.0000	0.0000	0.0000	-	0.0065	0.0099	0.0265	0.0368	0.0331	0.0163	0.0163	0.0163	0.0730
Psc#1	0.0065	0.0132	0.0065	0.0065	0.0097	0.0065	0.0065	0.0065	0.0065	-	0.0032	0.0334	0.0368	0.0335	0.0096	0.0096	0.0096	0.0699
Psc#3	0.0098	0.0165	0.0098	0.0098	0.0130	0.0098	0.0098	0.0098	0.0098	0.0032	-	0.0369	0.0404	0.0370	0.0130	0.0130	0.0130	0.0738
Pss#5	0.0258	0.0324	0.0257	0.0257	0.0290	0.0257	0.0257	0.0257	0.0257	0.0322	0.0354	-	0.0440	0.0131	0.0366	0.0366	0.0366	0.0857
Pse#4	0.0355	0.0423	0.0354	0.0354	0.0387	0.0354	0.0354	0.0354	0.0354	0.0354	0.0387	0.0419	-	0.0439	0.0400	0.0400	0.0400	0.0699
Ps#6	0.0322	0.0418	0.0320	0.0320	0.0352	0.0320	0.0320	0.0320	0.0320	0.0324	0.0356	0.0129	0.0419	-	0.0364	0.0364	0.0364	0.0884
Ps#7	0.0160	0.0256	0.0160	0.0160	0.0192	0.0160	0.0160	0.0160	0.0160	0.0096	0.0128	0.0353	0.0385	0.0351	-	0.0000	0.0000	0.0802
Ps#8	0.0160	0.0256	0.0160	0.0160	0.0192	0.0160	0.0160	0.0160	0.0160	0.0096	0.0128	0.0353	0.0385	0.0351	0.0000	-	0.0000	0.0802
Ps#9	0.0160	0.0256	0.0160	0.0160	0.0192	0.0160	0.0160	0.0160	0.0160	0.0096	0.0128	0.0353	0.0385	0.0351	0.0000	0.0000	-	0.0802
Ppi#1	0.0686	0.0784	0.0684	0.0684	0.0717	0.0684	0.0684	0.0684	0.0684	0.0657	0.0690	0.0789	0.0657	0.0815	0.0747	0.0747	0.0747	-

1999; Bombi & Bologna, unpubl. data). In this case, the mitochondrial haplotypes assign them unequivocally to *P. s. campestris*. Ps#7, Ps#8, and Ps#9 plotted rather unequivocally within the *P. s. campestris* clade; they were linked to the Italian sequences, although with weak bootstrap support (MP: 38%, NJ: 60%).

Introduced American populations (KS and NY)

These introduced populations have been traditionally ascribed to *campestris*, on the basis of external morphology. Given the problems that have arisen with morphological subspecific attribution of some *sicula* specimens from native populations, we checked the introduced American populations by independent markers. The samples from the two surviving ones (KS and NY), plotted in an American clade with relatively good bootstrap support (MP: 70%, NJ: 77%). They were included in a *P. s. campestris* clade evident from the trees, with fair-good (MP: 60%) to good (NJ: 76%) bootstrap support. The origin from captive stocks suggests the possibility of interbreeding between original specimens of different origin, and even mixing of gene pools from different subspecies cannot be ruled out. Thus, what we have scored is mitochondrial affinity. A definitive check with nuclear markers would help define the matter.

Podarcis sicula has been the object of several of the first biochemical studies in population genetics (Nevo *et al.*, 1972; Gorman *et al.*, 1975). From the ecological point of view, *P. sicula* has proven that it can spread very rapidly as well as successfully outcompete autochthonous lizards after introduction, as in Aeolian islands (Sicily), where the native *P. raffonei* is severely endangered (Capula, 1992, 1994). Both of the assayed American populations (KS and NY) have also spread rapidly. There are no native lizards on Long Island, so the NY population does not compete with other lizards until it spreads considerably. On the other hand, there are several potentially competing lizard species in Shawnee County KS, and Great Plains skinks (*Eumeces obsoletus*) have been observed chasing *P. sicula* in areas where the *P. sicula* have moved outside highly urbanized habitats (Miller, pers. commun.).

The original native populations of *P. sicula* show relatively high levels of genetic variation, as scored by Capula (1994), and this is confirmed by the mitochondrial patterns in the present research. On the basis of the few specimens assayed, the two introduced populations display a very low level of nucleotide variation. Both their origin from captive stocks and possible bottleneck or founder effects from the time of their release could explain this pattern. Future research should be addressed to the study of the competition with autochthonous lizards, as well as to the effect of this low genetic variation.

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