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Genetic variation and differentiation in the lizard, *Podarcis wagleriana* (Reptilia: Lacertidae)

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The electrophoretic variation at 26 presumptive gene loci was investigated in populations of *Podarcis wagleriana* from Sicily, the Aegadian Islands, and the Aeolian Islands. For interspecific comparison, samples of the closely related lizard *P. sicula* from the same geographic area were also used. Population heterogeneity analyses carried out by the estimation of *F*-statistics and Nei's standard genetic distance, showed a high genetic homogeneity within *P. sicula*, but a noticeable genetic differentiation within *P. wagleriana*. In the latter species, Nei's *D* ranged from 0 to 0.212, and this is because the Aeolian populations were quite distinct from those inhabiting Sicily and the Aegadian Islands. Fixed differences identified at three loci (*Ck*, *Ada*, *Gp-4*) contributed to a relatively high value of Nei's standard genetic distance between the two population groups ($D = 0.147$). This value is very similar to those found comparing pairs of well-recognized biological species included in the genera *Podarcis* and *Lacerta*. Estimation of the time of evolutionary divergence shows that the Aeolian and Sicilian populations of *P. wagleriana* have been isolated geographically for a long time (0.7 Myr according to Nei's formula; 2 Myr according to Sarich's calibration), indicating evolutionary divergence at the species level. Based on genetic and biogeographic data, it is suggested the recognition of full specific status for the Aeolian populations, for which the name *P. raffonei* comb. nova (Aeolian wall lizard) is proposed. Electrophoretic data and comparative species distributions suggest that (1) *Podarcis sicula* recently colonized the Aeolian Islands, and (2) it has competed successfully with *P. raffonei* in this area, greatly reducing the range of the latter and causing the extinction of most of its populations. In fact, *P. sicula* is widespread in the Aeolian Archipelago, while *P. raffonei* is confined at present to one large island (Vulcano) and three tiny islands (Strombolicchio, Scoglio Faraglione, La Canna). This can be considered a classic example of competitive exclusion of a native form (*P. raffonei*) by a species accidentally introduced by man (*P. sicula*).

ADDITIONAL KEY WORDS:—*Podarcis raffonei* – *Podarcis sicula* – *Podarcis wagleriana* – allozyme electrophoresis – population heterogeneity – gene flow – genetic divergence – biogeography.

CONTENTS

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INTRODUCTION

Podarcis wagleriana Gistel, 1868 is a lacertid lizard inhabiting Sicily (with the exception of the NE part) and some satellite islands, i.e. the Aegadian Islands (Levanzo, Favignana, Marettimo), the Stagnone Islands (Isola Grande), and the Aeolian Islands (Vulcano, Strombolicchio) (Klemmer, 1956; Lanza, 1973; Böhme 1986; Capula *et al.*, 1987; Capula, 1990). The species is regarded as polytypic, and according to Capula (1990) it is represented by the following subspecies: *P. w. wagleriana* Gistel, 1868 (Sicily, Levanzo, Favignana, and Isola Grande dello Stagnone); *P. w. marettimensis* (Klemmer, 1956) (Marettimo); *P. w. antoninoi* (Mertens, 1955) (Vulcano); *P. w. raffonei* (Mertens, 1952) (Strombolicchio, near Stromboli).

In Sicily and on some minor Sicilian islands (Aegadian, Stagnone and Aeolian) *Podarcis wagleriana* is broadly sympatric with *P. sicula* (Rafinesque, 1810) (Capula, 1990, 1992). At the morphological level, identification of *Podarcis wagleriana* and *P. sicula* in the Sicilian localities where they coexist may be difficult. In fact, these two lacertid lizards are quite similar in most anatomical features, differing slightly only in colour pattern (see Lanza, 1968; Arnold & Burton, 1978), and on some islands (e.g. Vulcano and Marettimo) they are known to hybridize (Capula, 1993). On the other hand, the detection of diagnostic electrophoretic loci between the two species allows a correct identification of all invididuals, either in overlapping areas or in allopatry (see Capula *et al.*, 1987; Capula, 1990, 1993).

In the present paper, allozyme variation in *Podarcis wagleriana* was studied by means of horizontal starch gel electrophoresis. This investigation highlighted a substantial genetic differentiation between Sicilian and Aeolian samples. Since the level of genetic divergence between the studied populations is an indirect indication of their isolation (see e.g. Sarich, 1977; Sbordoni *et al.*, 1990), the time elapsed since the presumed geographic isolation was estimated by inferring it from genetic distance data. Divergence times were then compared with palaeogeographic evidence.

Here are presented genetic and biogeographic data supporting the hypothesis that these two population groups, currently regarded as *P. wagleriana*, are genetically distinct and have been isolated geographically for a long time, indicating evolutionary divergence at the species level.

MATERIAL AND METHODS

Sampling

Samples used in this study, including *Podarcis wagleriana* and the related *P. sicula* (the latter employed for interspecific comparison only), were obtained from 14 localities, including five from Sicily, three from the Aegadian Islands

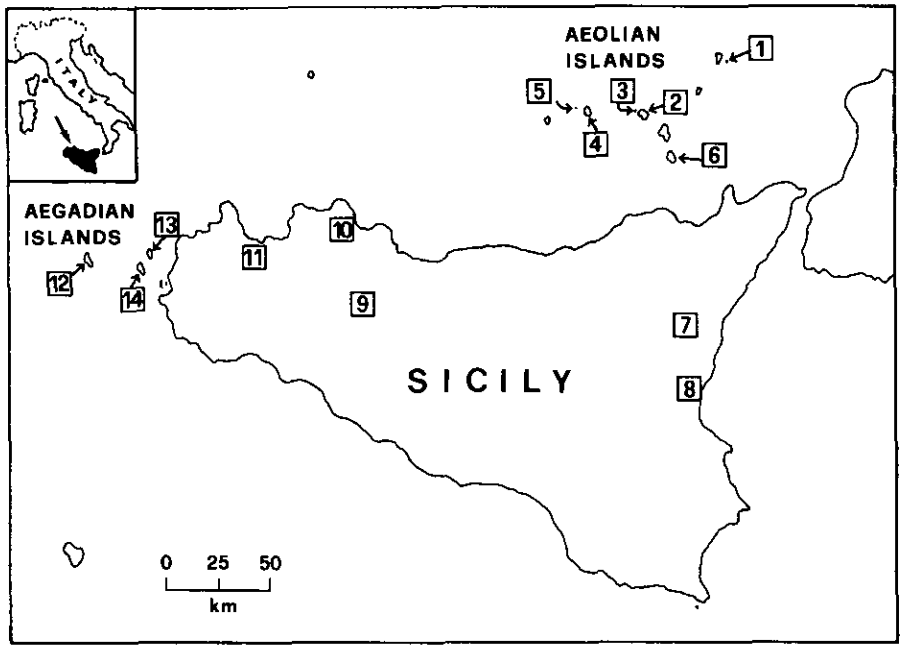

Figure 1. Map of Sicily, Aeolian Islands and Aegadian Islands showing localities from which *Podarcis* were examined biochemically. (1) Strombolicchio; (2) Salina; (3) Scoglio Faraglione; (4) Filicudi; (5) La Canna; (6) Vulcano; (7) Linguaglossa; (8) Primosole; (9) Ficuzza; (10) Palermo; (11) Castellammare del Golfo; (12) Marettimo; (13) Levanzo; (14) Favignana. Insert shows location of the study area.

and six from the Aeolian Islands (Fig. 1). Geographic origin and the number of specimens analysed per population are indicated in Table 1.

All individuals were collected in the field and transported to the laboratory. Specimens were anaesthetized with ethyl ether and then dissected. Homogenates were stored below $-70°C$.

TABLE 1. Geographic and collecting data for *Podarcis* used in the present study (nomenclature according to current classification)

Population	Species	Sample size	Locality
1.	*P. wagleriana wagleriana*	10	Primosole (Sicily)
2.	*P. wagleriana wagleriana*	10	Castellammare del Golfo (Sicily)
3.	*P. wagleriana wagleriana*	10	Palermo (Sicily)
4.	*P. wagleriana wagleriana*	8	Ficuzza (Sicily)
5.	*P. wagleriana wagleriana*	18	Favignana (Aegadian Islands)
6.	*P. wagleriana wagleriana*	8	Levanzo (Aegadian Islands)
7.	*P. wagleriana marettimensis*	32	Marettimo (Aegadian Islands)
8.	*P. wagleriana raffonei*	11	Strombolicchio (Aeolian Islands)
9.	*P. wagleriana antoninoi*	14	Vulcano (Aeolian Islands)
10.	*P. sicula cucchiarai*	4	La Canna (Aeolian Islands)
11.	*P. sicula alvearioi*	5	Scoglio Faraglione (Aeolian Islands)
12.	*P. sicula sicula*	20	Salina (Aeolian Islands)
13.	*P. sicula sicula*	21	Filicudi (Aeolian Islands)
14.	*P. sicula sicula*	7	Linguaglossa (Sicily)
15.	*P. sicula sicula*	8	Palermo (Sicily)
16.	*P. sicula sicula*	11	Favignana (Aegadian Islands)

Electrophoresis

Standard horizontal starch gel electrophoresis was performed on leg muscle tissue, which was crushed in distilled water. Homogenates from single individuals were absorbed into 5 × 5 mm pieces of chromatography paper (Whatman 3 MM) and inserted in 10% Connaught starch gel trays. Electrophoresis was carried out at 7–9 V/cm for 4–6 h at 5°C. After the run, gels were sliced in two parts and each slice was stained for a specific enzyme. Gene products for the following 23 presumptive enzyme loci were analysed: *αGpd*, *Ldh-1*, *Ldh-2*, *Mdh-1*, *Mdh-2*, *Me-1*, *Me-2*, *Idh-1*, *Idh-2*, *6Pgd*, *Gapd*, *Sod*, *Np*, *Got-1*, *Got-2*, *Ck*, *Ak*, *Pgm-1*, *Pgm-2*, *Ada*, *Ca-2*, *Mpi*, *Gpi* (enzymes codes are according to Richardson, Baverstock & Adams, 1986). In addition, three unidentified non-enzymatic proteins, i.e. *Gp-1*, *Gp-2*, *Gp-4*, were studied. The buffer systems used and electrophoretic procedures are given in Tables 2 and 3 respectively. The staining techniques used were those described by Capula (1990).

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 Palermo (Sicily), indicated as 100(> 100 = faster mobility; < 100 = slower mobility).

Analysis

The genetic variability of populations was estimated using the following parameters: observed mean heterozygosity per locus (H_o); expected mean heterozygosity per locus (H_e) (unbiased estimate, Nei, 1978); proportion of polymorphic loci, at the 99% level (P); mean number of alleles per locus (A).

The distribution of genetic variation within and among populations was

TABLE 2. Buffer systems. Analytical grade reagents per litre; pH at room temperature

Buffer system	Electrodes	Gel
1. Discontinuous Tris/citrate (Na) (Poulik, 1957)	0.3 M sodium borate, pH 8.2 (18.55 g boric acid, 2.40 g NaOH)	0.076 M Tris/0.005 M citric acid, pH 8.7 (9.21 g Tris, 1.05 g monohydrate citric acid)
2. Continuous Tris/citrate (Selander *et al.*, 1971)	0.687 M Tris/0.157 M citric acid, pH 8 (83.2 g Tris, 30 g monohydrate citric acid)	0.023 M Tris/0.005 M citric acid, pH 8 (2.77 g Tris, 1.10 g monohydrate citric acid)
3. Tris/versene borate (Brewer & Sing, 1970)	0.21 M Tris/0.15 M boric acid/0.006 M EDTA, pH 8 (25.4 g Tris, 9.24 g boric acid, 2.20 g EDTA)	0.021 M Tris/0.02 M boric acid/0.007 M EDTA, pH 8 (2.5 g Tris, 1.24 g boric acid, 0.25 g EDTA)
4. Phosphate/citrate (Harris, 1966)	0.15 M tri-sodium citrate/0.24 M sodium hydrogen phosphate, pH 6.3 (44.11 g sodium citrate, 33.12 g NaH_2PO_4)	electrode buffer diluted 1 : 40, pH 6.3
5. Tris/maleate (modified from Brewer & Sing, 1970)	0.01 M Tris/0.1 M maleic acid/0.01 M EDTA/0.015 $MgCl_2$/0.125 M NaOH, pH 7.2 (12.11 g Tris, 11.61 g maleic acid, 3.72 g EDTA, 2.03 g $MgCl_2$, 5 g NaOH)	electrode buffer diluted 1 : 10, pH 7.4

TABLE 3. Enzymatic and non-enzymatic proteins examined and electrophoretic conditions employed. Enzymes are arranged by Enzyme Commission Number (EC)

Protein	Migration + = anodal − = cathodal	Buffer system	V/cm	Time (h)	References
Glycerol-3-phosphate dehydrogenase αGPD (EC 1.1.1.8)	+	4	8	6	Ayala *et al.*, 1972
Lactate dehydrogenase LDH (EC 1.1.1.27)	+	5	7	6	Brewer & Sing, 1970
Malate dehydrogenase MDH (EC 1.1.1.37)	+	4	8	5	Shaw & Prasad, 1970
Malic enzyme ME (EC 1.1.1.40)	+	2	8	6	Ayala *et al.*, 1972
Isocitrate dehydrogenase IDH (EC 1.1.1.42)	+	4	8	5	Shaw & Prasad, 1970
6–Phosphogluconate dehydrogenase 6PGD (EC 1.1.1.44)	+	4	8	6	Shaw & Prasad, 1970
Glyceraldehyde-3-phosphate dehydrogenase GAPD (EC 1.2.1.12)	+	3	7	6	Ayala *et al.*, 1972
Superoxide dismutase SOD (EC 1.15.1.1)	+	2	8	5	Selander *et al.*, 1971
Purine nucleoside phosphorylase NP (EC 2.4.2.1)	+	3	8	5	Harris & Hopkinson, 1976
Glutamate-oxaloacetate transaminase GOT (EC 2.6.1.1)	±	4	9	6	Selander *et al.*, 1971
Creatine kinase CK (EC 2.7.3.2)	+	2	8	5	Ayala *et al.*, 1972
Adenylate kinase AK (EC 2.7.4.3)	+	2	8	5	Ayala *et al.*, 1972
Phosphoglucomutase PGM (EC 2.7.5.1)	+	5	8	6	Brewer & Sing, 1970
Adenosine deaminase ADA (EC 3.5.4.4)	+	2	7	4	Harris & Hopkinson, 1976
Carbonate dehydratase CA (EC 4.2.1.1)	+	3	8	4	Harris & Hopkinson, 1976
Mannose phosphate isomerase MPI (EC 5.3.1.8)	+	3	8	4	Harris & Hopkinson, 1976
Glucose-phosphate isomerase GPI (EC 5.3.1.9)	−	4	8	6	Selander *et al.*, 1971
General proteins GP	+	1	8	4	Scott & McClelland, 1975

assessed using Wright's *F*-statistics (Wright, 1965). The three *F*-statistics are interrelated so that

$$F_{ST} = (F_{IT} - F_{IS})/(1 - F_{IS})$$

F_{IS} and F_{IT} are measures of deviation from Hardy-Weinberg proportions within subdivisions and the total sample respectively. F_{ST} is a measure of genetic differentiation among subdivisions. Statistical significance of F_{ST} was tested by the χ^2-test: $\chi^2 = 2N_t F_{ST}(k-1)$, with $(k-1)(s-1)$ degrees of freedom, where k is the number of alleles and s is the number of subdivisions (Workman & Niswander, 1970).

The genetic relationships among the studied populations were evaluated using Nei's (1972) standard genetic identity (I) and standard genetic distance (D). We did not use Nei's (1978) unbiased genetic identity and distance (I and D modified for small samples) because we did not score a sufficient number of gene loci (see Nei, 1978). All genetic variability, *F*-statistics, and genetic distance measures were calculated by the computer program BIOSYS-1 (Swofford & Selander, 1981).

Genotype data at 26 electrophoretic loci were analysed for rates of gene flow following the Slatkin's (1985) model. To estimate gene flow (Nm) we used the Slatkin's formula:

$$\ln[\bar{p}(1)] = -0.505 \ln(Nm) - 2.44$$

Because the constants in the above formula are for sample sizes of 25, values of *Nm* were adjusted for sample size following Slatkin (1985).

Estimation of phenetic relationships among populations was obtained by generating phenograms of all samples by means of UPGMA cluster analysis based on the matrix of Nei's genetic distances (Sneath & Sokal, 1973).

Nonparametric multidimensional scaling (MDS), generated on the basis of Rogers' (1972) genetic distances, which are metrics, was performed in order to obtain the ordination of populations in a multidimensional space (Nei's genetic distance measure is not a metric, i.e. it violates the triangle inequality, and thus cannot be interpreted as a measure of evolutionary path length; see Rogers, 1984). This multivariate ordination procedure constructs a configuration of points in the Euclidean space, which reflects the relationships between a set of populations (Dunn & Everitt, 1982). MDS requires no more than ordinal relations in the original distance matrix, maintaining a close agreement with the initial data matrix (Shepard, 1962). A special advantage of the nonparametric MDS method is that it seems better than principal component analysis (PCA) in giving balance between the large intercluster distances and the fine differences between members of a given cluster (Rohlf, 1970).

The time of evolutionary divergence between taxa was estimated from Nei's (1972) genetic distance data using (1) Nei's (1975) formula: $t = 5 \times 10^6 D$, which is based on the assumption that for small values of $D (D < 1)$ genetic distances seem linearly related to time, and (2) Sarich's (1977) calibration (corrected by Maxson & Maxson, 1979): $1D = 14 \times 10^6$ years, which calibrates divergence times according to the different contribution of the gene loci studied (fast and slow evolving loci) to the genetic distance estimates. Both Nei's formula and Sarich's calibration are usually used to test the 'molecular clock hypothesis', being rough estimates of the time required by two taxa to undergo divergent evolution (see e.g. Thorpe, 1982). Isolation times estimated by these two methods were then compared with palaeogeographic data in order to assess which of the predicted divergence times were roughly in accord with available geological evidence.

RESULTS

Of the 26 presumptive gene loci analysed, eight (31%) were found monomorphic and fixed for the same allele in all the studied samples (*Ldh-2*, *Mdh-1*, *Mdh-2*, *Sod*, *Np*, *Got-2*, *Ak*, *Pgm-1*). The allele frequencies at the other 18 variable loci are given in Table 4. Four loci (15%) were locally (≥ 6 populations) and strongly polymorphic (*Me-1*, *Idh-1*, *Ca-2*, *Mpi*); eight loci (31%) were locally (≤ 2 populations) and in most cases weakly polymorphic (αGpd, *Ldh-1*, *Me-2*, *Idh-2*, *6Pgd*, *Pgm-2*, *Ada*, *Gpi*).

As to the diagnostic loci (at the 99% level) between *P. sicula* and *P. wagleriana*, a very particular situation was found. Six loci (23%) displayed fixation of alternative alleles between *P. sicula* and Sicilian and Aegadian populations of *P. wagleriana* (*Gapd*, *Got-1*, *Ck*, *Gp-1*, *Gp-2*, *Gp-4*), while only four out of these loci, i.e. *Gapd*, *Got-1*, *Gp-1*, *Gp-2*, were found to be fixed for alternative alleles between *P. sicula* and Aeolian samples of *P. wagleriana*, these latter apparently sharing with *P. sicula* the same electrophoretic allele at the *Ck* and *Gp-4* loci (see Table 4). On the other hand, the *Idh-1* locus displayed fixation of alternative

TABLE 4. Allele frequencies at 18 variable loci in *Podarcis* populations from Sicily, Aeolian Islands, and Aegadian Islands. For geographic origin of populations see Table 1

Locus	Allele	Population 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
αGpd																	
	95	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	0.937	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-1																	
	80	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me-1																	
	96	0.000	0.000	0.125	0.000	0.062	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.375	0.000	0.071	0.000	0.000	0.000	0.000	0.971	0.850	1.000	1.000	1.000
	104	1.000	0.875	0.875	0.917	0.563	1.000	0.929	1.000	1.000	1.000	1.000	0.029	0.150	0.000	0.000	0.000
	108	0.000	0.125	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Me-2																	
	100	1.000	1.000	1.000	1.000	0.917	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	110	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Idh-1																	
	94	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.000	0.350	0.200	0.000	0.694	0.187	0.274	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
	106	0.917	0.650	0.800	1.000	0.306	0.813	0.726	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
Idh-2																	
	96	0.000	0.000	0.050	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	0.950	1.000	1.000	0.937	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6Pgd																	
	95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000	0.929	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	110	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gapd																	
	94	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
Got-1																	
	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
	105	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
Ck																	
	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	110	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 4—*continued*

Locus	Allele	Population 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
Pgm-2																	
	92	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	0.950	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ada																	
	92	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.937	0.625	1.000	1.000	1.000
	103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	110	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.375	0.000	0.000	0.000
Ca-2																	
	90	0.000	0.100	0.000	0.062	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	0.750	0.950	0.938	0.656	0.937	0.793	0.833	0.833	1.000	0.900	1.000	1.000	1.000	1.000	1.000
	110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.167	0.000	0.100	0.000	0.000	0.000	0.000	0.000
	115	0.000	0.150	0.050	0.000	0.281	0.063	0.207	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mpi																	
	96	0.000	0.150	0.300	0.125	0.166	0.000	0.190	0.000	0.000	0.000	0.000	0.325	0.088	0.000	0.937	0.000
	100	1.000	0.850	0.650	0.875	0.778	1.000	0.741	0.944	1.000	1.000	1.000	0.675	0.912	1.000	0.063	1.000
	104	0.000	0.000	0.050	0.000	0.056	0.000	0.069	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gpi																	
	90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.937	1.000
Gp-1																	
	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
	105	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
Gp-2																	
	95	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
Gp-4																	
	100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	110	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

alleles between *P. sicula* (*Idh-1*^{100}) and *P. wagleriana* from the Aeolian Islands (*Idh-1*^{106}), while it showed a high polymorphism in Sicilian and Aegadian samples of *P. wagleriana*. In these samples, *Idh-1*^{106} was found at high frequencies (≥ 0.65, not including the sample from Favignana), while *Idh-1*^{100} appeared with lower frequencies (≤ 0.35, not including the sample from Favignana).

Unexpectedly, three loci (*Ck*, *Ada*, *Gp-4*) showed fixation of alternative alleles between populations of *P. wagleriana* from the Aeolian Islands and *P. wagleriana* populations from Sicily and the Aegadian Islands. Moreover, populations of *P. wagleriana* from the Aeolian Islands were characterized by a unique electrophoretic allele (*Ca-2*^{110}), present at a noticeable frequency (0.17), and, in one case (sample from Strombolicchio), showed fixation of another unique allele (*Pgm-2*^{105}). These results clearly indicate that there is a noticeable level of genetic differentiation between Aeolian and Sicilian populations of *P. wagleriana*.

Surprisingly, samples from La Canna and Scoglio Faraglione (Aeolian Islands), though currently assigned to *P. sicula* (see Mertens, 1955; Di Palma, 1980), were found to be genetically highly differentiated from this latter species, while being very close to the Aeolian populations of *P. wagleriana* (Strombolicchio and Vulcano), with almost identical allele frequencies at 25 (96%) out of 26 loci (see Table 4).

Deviations from Hardy-Weinberg equilibrium owing to heterozygote deficiencies were found in the following populations (in parentheses) and loci: Castellammare del Golfo (2) *Ca-2* ($P < 0.01$); Favignana (5), *Me-1* ($P = 0.02$); Filicudi (13), *Mpi* ($P < 0.002$).

Genetic variability

The considered genetic variability parameters (H_o, H_e, P, A) are given in Table 5. The levels of polymorphism (P) and heterozygosity (H_o) detected in

TABLE 5. Genetic variability parameters in *Podarcis* populations from Sicily, Aeolian Islands, and Aegadian Islands. 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; Nei, 1978) (S.E. = standard error)

Population	Mssl	A	P	H_o	(S.E.)	H_e	(S.E.)
1.	8.0	1.0	3.8	0.006	(0.006)	0.006	(0.006)
2.	9.8	1.2	19.2	0.063	(0.031)	0.058	(0.027)
3.	9.4	1.3	26.9	0.060	(0.029)	0.058	(0.025)
4.	7.6	1.1	11.5	0.021	(0.012)	0.020	(0.012)
5.	15.0	1.3	19.2	0.076	(0.035)	0.078	(0.034)
6.	6.6	1.1	11.5	0.014	(0.008)	0.022	(0.014)
7.	28.3	1.2	19.2	0.057	(0.026)	0.054	(0.025)
8.	9.4	1.1	7.7	0.017	(0.013)	0.016	(0.012)
9.	12.1	1.1	7.7	0.018	(0.014)	0.016	(0.012)
10.	4.0	1.0	0.0	0.000		0.000	
11.	4.7	1.0	3.8	0.008	(0.008)	0.008	(0.008)
12.	15.7	1.1	11.5	0.021	(0.014)	0.024	(0.018)
13.	16.5	1.1	11.5	0.036	(0.025)	0.036	(0.022)
14.	6.5	1.0	0.0	0.000		0.000	
15.	7.3	1.1	7.7	0.010	(0.007)	0.010	(0.007)
16.	9.6	1.0	0.0	0.000		0.000	

P. wagleriana populations from Sicily ($P = 0.15$; $H_o = 0.037$) and the Aegadian Islands ($P = 0.17$; $H_o = 0.049$) were similar both to the average ones calculated by Nevo (1978) for 17 species of reptiles ($P = 0.22$; $H_o = 0.047$), and the average ones calculated by Capula (1990) for nine species of *Podarcis* ($P = 0.13$; $H_o = 0.053$). The Aeolian samples of *P. wagleriana* showed noticeably lower values of polymorphism and heterozygosity ($P = 0.05$; $H_o = 0.011$). The severe reduction in genetic variability pointed out in these samples could be due either to genetic drift phenomena or strong directional selection, as supported by the fact that three out of four populations of *P. wagleriana* known to occur in the Aeolian Archipelago inhabit tiny volcanic islands (Strombolicchio, Scoglio Faraglione, La Canna), each characterized by a very limited area (< 0.02 km^2). Unfortunately, the present data do not permit us to distinguish between the drift and selection hypotheses.

The levels of polymorphism and heterozygosity are below the mean for the species of reptiles analysed by Nevo (1978) and Capula (1990) (see above) also in the samples of *P. sicula* ($P = 0.06$; $H_o = 0.013$). In the case of *P. sicula* populations inhabiting satellite islands, however, the observed low genetic variability could be due to founder effects (*sensu* Mayr, 1982; Barton & Charlesworth, 1984), the Aeolian and Aegadian populations of the species probably having originated from episodes of accidental anthropogenic introduction in protohistorical or historical times (Lanza, 1973; Capula *et al.*, 1987; Capula, 1990) (see Discussion).

Population heterogeneity

The mean F_{ST} among Sicilian and Aegadian populations of *Podarcis wagleriana* was 0.153 (Table 6). Five of the 10 single-locus F_{ST} values were statistically significant, suggesting some genetic differentiation among populations. However, it must be stressed that *Idh-1* had a much higher F_{ST} than all other loci. If this locus is removed because of possible scoring errors, the mean F_{ST} reduces to 0.113. Mean F_{ST} values are also given for all *P. wagleriana* samples and the Total (*P. wagleriana* plus *P. sicula*) (Table 6). Adding the Aeolian samples of *P. wagleriana* increases the F_{ST} to a very high level (0.625), suggesting that the Aeolian populations are highly differentiated from both the Sicilian and Aegadian ones. Including the populations of *P. sicula* increases F_{ST} (0.872) further, indicating that the latter species is genetically quite differentiated from *P. wagleriana*. F_{IS} values remain low regardless of which populations are included, presumably because Hardy-Weinberg proportions are maintained within populations by random mating.

The values of Nei's genetic identity and genetic distance for each pairwise comparison are shown in Table 7. A high genetic homogeneity was found within *P. sicula*. The values of Nei's standard genetic distance observed between the populations of this species ranged from $D = 0$ to $D = 0.035$ (average $D = 0.015$). The Aeolian populations of *P. sicula* (Filicudi and Salina) were genetically very similar to each other and to those from Sicily and the Aegadian Islands (average $D = 0.012$).

As reported above, the lizards from Scoglio Faraglione and La Canna, though currently assigned to *P. sicula* (Henle & Klaver, 1986), were found to be genetically highly differentiated from all other populations of this species

TABLE 6. Summary of *F*-statistics at ten loci for *Podarcis wagleriana* populations from Sicily and Aegadian Islands. The mean across all loci is also given for *P. wagleriana* as a whole (S = Sicilian and Aegadian populations; A = Aeolian populations, which in the present paper were recognized as *P. raffonei*), and Total (all *Podarcis* samples studied). Asterisks denote statistical significance for F_{ST} as determined by the χ^2-test (see text). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

Locus	F_{IS}	F_{IT}	F_{ST}
αGpd	−0.067	−0.009	0.054
Ldh-1	−0.053	−0.007	0.043
Me-1	0.160	0.314	0.182***
Me-2	−0.091	−0.012	0.072*
Idh-1	−0.027	0.219	0.240***
Idh-2	−0.060	−0.016	0.042
6Pgd	−0.053	−0.007	0.043
Pgm-2	−0.053	−0.007	0.043
Ca-2	−0.046	0.062	0.103***
Mpi	−0.237	0.123	0.092***
MEAN	−0.052	0.108	0.153
MEAN for:			
P. wagleriana (S+A)	−0.064	0.601	0.625
Total	−0.052	0.866	0.872

(average $D = 0.321$). These two samples shared the same allele frequencies (see Table 4), and were very close both to *P. wagleriana antoninoi* from Vulcano and *P. wagleriana raffonei* from Strombolicchio (average $D = 0.020$).

Within *P. wagleriana*, high values of genetic distance were found, D ranging from 0 to 0.212 (average $D = 0.080$). This is because the Aeolian populations of the Sicilian wall lizard (including those from Scoglio Faraglione and La Canna,

TABLE 7. Values of Nei's (1972) standard genetic identity (above the diagonal) and standard genetic distance (below the diagonal) among populations of *Podarcis* from Sicily, Aeolian Islands, and Aegadian Islands

Population	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
1.	—	0.993	0.994	0.999	0.971	0.999	0.994	0.843	0.882	0.884	0.883	0.652	0.684	0.656	0.620	0.656
2.	0.007	—	0.996	0.994	0.989	0.996	0.998	0.833	0.873	0.873	0.873	0.669	0.698	0.669	0.643	0.669
3.	0.006	0.005	—	0.996	0.981	0.995	0.997	0.836	0.873	0.875	0.874	0.663	0.689	0.658	0.646	0.658
4.	0.001	0.006	0.004	—	0.972	0.997	0.995	0.842	0.881	0.883	0.882	0.652	0.682	0.653	0.626	0.653
5.	0.030	0.011	0.019	0.029	—	0.979	0.987	0.809	0.848	0.849	0.849	0.697	0.723	0.697	0.675	0.697
6.	0.001	0.004	0.005	0.003	0.022	—	0.996	0.841	0.880	0.882	0.881	0.659	0.692	0.663	0.627	0.663
7.	0.006	0.002	0.003	0.005	0.013	0.004	—	0.835	0.874	0.875	0.875	0.667	0.695	0.665	0.645	0.665
8.	0.171	0.183	0.179	0.172	0.212	0.173	0.181	—	0.961	0.960	0.961	0.686	0.704	0.689	0.655	0.689
9.	0.126	0.136	0.136	0.127	0.164	0.128	0.135	0.040	—	0.999	1.000	0.725	0.743	0.727	0.692	0.727
10.	0.123	0.135	0.134	0.125	0.164	0.126	0.134	0.041	0.001	—	1.000	0.728	0.746	0.731	0.695	0.731
11.	0.124	0.135	0.134	0.126	0.164	0.126	0.134	0.040	0.000	0.000	—	0.727	0.745	0.729	0.694	0.729
12.	0.428	0.403	0.411	0.428	0.360	0.416	0.406	0.377	0.322	0.317	0.319	—	0.992	0.996	0.985	0.996
13.	0.379	0.359	0.372	0.383	0.324	0.368	0.364	0.352	0.298	0.293	0.295	0.008	—	0.993	0.965	0.993
14.	0.422	0.403	0.418	0.426	0.361	0.411	0.408	0.373	0.318	0.314	0.315	0.004	0.007	—	0.966	1.000
15.	0.478	0.441	0.437	0.469	0.394	0.467	0.439	0.423	0.369	0.363	0.365	0.015	0.035	0.035	—	0.966
16.	0.422	0.403	0.418	0.426	0.361	0.411	0.408	0.373	0.318	0.314	0.315	0.004	0.007	0.000	0.035	—

which were up to now considered as belonging to *P. sicula*), were quite differentiated from those inhabiting Sicily and the Aegadian Islands. Fixed differences identified at three loci (*Ck*, *Ada*, *Gp-4*) contributed to an average Nei's genetic distance of 0.147 between these population groups, indicating their reproductive isolation.

On the other hand, the samples of *P. wagleriana* from the Aegadian Islands were genetically very similar to the Sicilian ones, the genetic distance values found between these two population groups being quite low (average $D = 0.010$) and similar to those often detected within the genus *Podarcis* among local populations of the same species (see e.g. Mayer, 1981; Capula, 1990). Very low levels of genetic differentiation were also pointed out among the Sicilian samples of *P. wagleriana*, D ranging from 0.001 to 0.007.

As to the interspecific genetic distances, the value of Nei's genetic distance was relatively high when comparing *P. sicula* with the total sample of *P. wagleriana* ($D = 0.384$). However, it must be stressed that when comparisons were made considering two population groups of *P. wagleriana*, *P. sicula* was shown to be genetically nearer to the Aeolian populations of *P. wagleriana* (average $D = 0.337$) than to the Sicilian and Aegadian ones (average $D = 0.406$). This is because (1) fixation of alternative alleles between the Aeolian form of *P. wagleriana* and *P. sicula* occurred at four instead of six electrophoretic loci found to be diagnostic between Siculo-Aegadian populations of *P. wagleriana* and *P. sicula*, and (2) there was probably a limited introgression between the two lizards on the Aeolian Islands, as pointed out by Capula (1993).

Gene flow

The rate of gene flow (Nm) was calculated at various levels, from within each of the two major groups evidenced by genetic heterogeneity analysis to the total sample of *P. wagleriana*. The mean Nm among the Sicilian samples of *Podarcis wagleriana* was 4.30 [$\bar{p}(1) = 0.068$; Mean N = 9.5], while the mean Nm among Sicilian and Aegadian samples was 3.47 [$\bar{p}(1) = 0.063$; Mean N = 13.71]. These values are higher than that estimated by Slatkin (1985) for insular populations of *Podarcis melisellensis* ($Nm = 1.9$), and appear to be more than sufficient to prevent allelic fixation in different populations. As would be expected on the basis of both F-statistics and genetic distances data, including populations of the Sicilian wall lizard from the Aeolian Islands dramatically reduces Nm (0.50) [$\bar{p}(1) = 0.181$; Mean N = 11.82], thus indicating very little gene flow between Aeolian and the other populations of *P. wagleriana*. However, caution should be taken when interpreting or comparing Nm values obtained by the method employed in our study (see Slatkin, 1985). In fact, because accuracy at this scale is reduced under the model used, we can say little about the actual magnitude of gene flow other than that it is very low.

Cluster analysis and multidimensional scaling ordination

The UPGMA clustering procedure revealed three main clusters in the phenogram constructed on the basis of the matrix of Nei's standard genetic distances (Fig. 2). Cluster A contains the Sicilian and Aegadian populations of

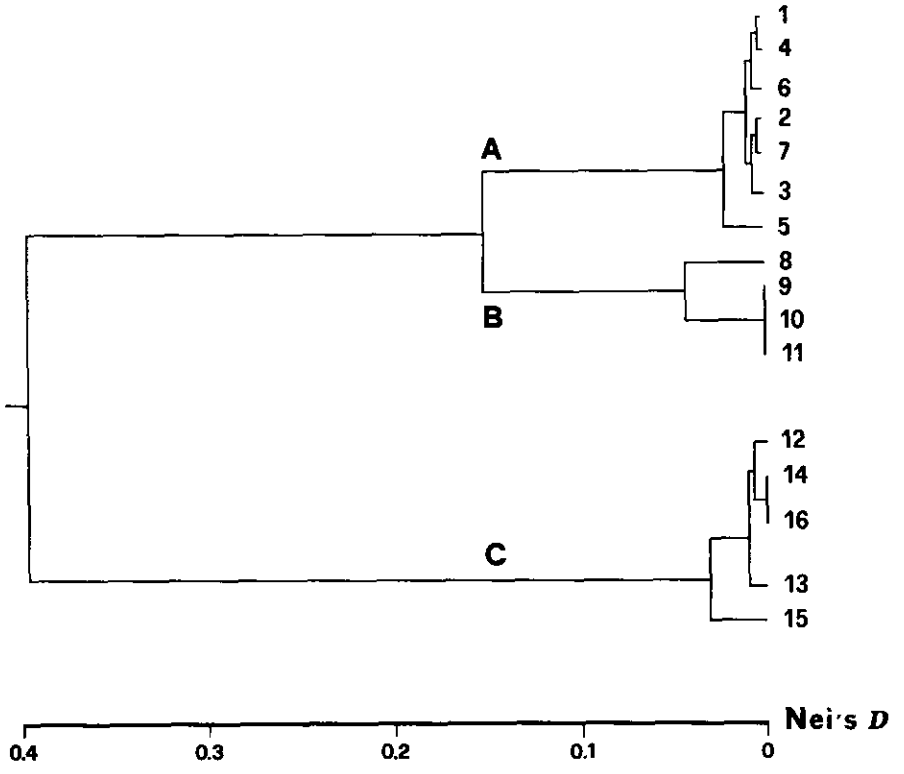

Figure 2. Phenogram generated by UPGMA cluster analysis based on Nei's (1972) standard genetic distances among *Podarcis* populations from Sicily, Aeolian Islands, and Aegadian Islands (Cophenetic correlation = 0.978). For geographic origin of populations see Table 1.

P. wagleriana, cluster B contains only *P. wagleriana* samples from the Aeolian Islands, and cluster C includes all five *P. sicula* populations. Within cluster B should be noted the existence of two subclusters, one including the closely grouped samples from Vulcano, Scoglio Faraglione and La Canna, the other containing only the sample from Strombolicchio, which is in fact characterized by a fixed electrophoretic allele ($Pgm\text{-}2^{105}$) not found in the other samples. Cophenetic correlation between the matrix of genetic distances and the derived phenogram was rather high (0.978).

The ordination of populations by multidimensional scaling (MDS) is shown in Fig. 3. Due to the very low value of stress found (0.0096), indicating a high degree of concordance of dissimilarities and distances (the stress function measures the extent to which the distances between points of the graphic representation are monotonic with the observed dissimilarities), only graphical representation on the planes defined by the two first dimensions was made. The results of the MDS ordination were remarkably similar to those obtained by the UPGMA clustering, the same three major groups (A, B, C) being clearly defined. The first coordinate axis separates (1) the populations of *P. sicula* from those of *P. wagleriana*, and (2) the Aeolian populations of *P. wagleriana* from the Sicilian and Aegadian ones; the second axis clearly distinguishes the Aeolian populations of *P. wagleriana* from the Sicilian and Aegadian ones.

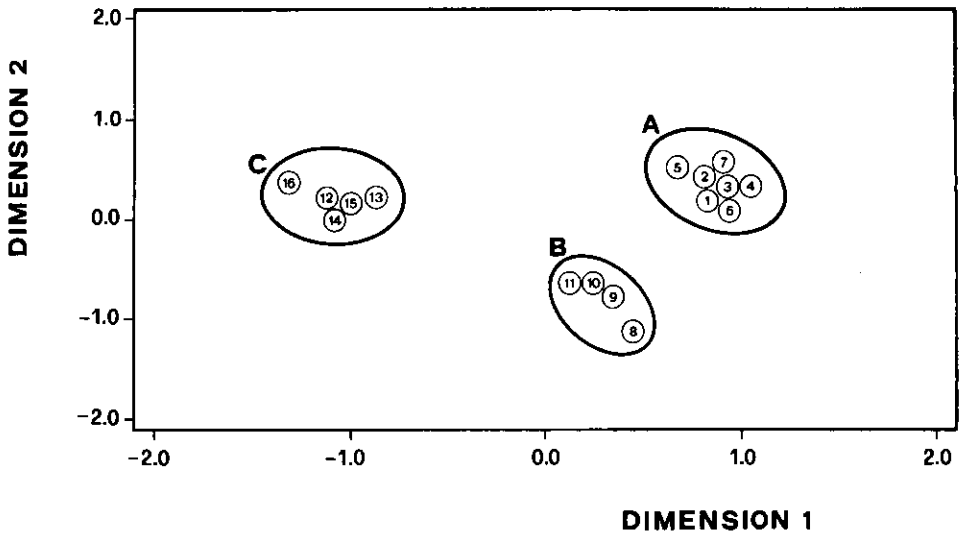

Figure 3. Bidimensional multidimensional scaling ordination of populations based on Rogers' (1972) genetic distances among *Podarcis* populations from Sicily, Aeolian Islands, and Aegadian Islands. Stress of final configuration = 0.0096.

DISCUSSION

Extent of divergence between populations

The results of our analyses on genetic heterogeneity and gene flow in *P. wagleriana* are consistent with the preliminary electrophoretic investigations previously carried out on the species (Capula, Nascetti & Bullini, 1988; Capula, 1990), and support the evidence that the Aeolian populations are genetically independent from those inhabiting Sicily and the Aegadian Islands.

Interspecific genetic differentiation has not been widely investigated within the Lacertidae. However, electrophoretically determined estimates of Nei's genetic distances are reported for some species of the genera *Podarcis* and *Lacerta*. In the genus *Podarcis*, Nei's *D* ranges from 0.07 (between *P. taurica* and *P. melisellensis*) to 0.65 (between *P. filfolensis* and *P. hispanica*) (Capula *et al.*, 1988; Capula, 1990). In the related genus *Lacerta*, Nei's *D* ranges from 0.13 (between *L. oxycephala* and *L. graeca*) to 1.70 (between *L. bedriagae* and *L. viridis*) (Mayer & Tiedemann, 1982; Busack, 1987). Although caution should be exercised in the interpretation of Nei's (1972) genetic distances, as genetic distances computed from differing numbers of electrophoretic loci among representatives of different taxa may not be directly comparable, they can provide an indication of the range of values to be expected between biological species within a genus (see e.g. Avise, 1974; Buth, 1984). Thus, the very low value of Nei's standard genetic distance found between the Aegadian and Sicilian samples of *P. wagleriana* ($D = 0.010$) suggests that these populations were geographically isolated in geologically recent times and represent a single systematic, as well as genetic, unit. The time of genetic divergence estimated on the basis of the *D* value according to Nei's (1975) formula seems to go back to the Upper Pleistocene (0.05 Myr); this is in good agreement with palaeogeographic evidence and would confirm the hypothesis of a recent isolation of the two population groups.

In fact, it is well known that at least two islands of the Aegadian Archipelago, i.e. Favignana and Levanzo, were linked several times to Sicily either during the Middle Pleistocene (e.g. between the Calabrian and Sicilian periods) (Ruggieri, 1973) or during the Upper Pleistocene (Würm), when they were colonized by large continental mammals from the main island (Malatesta, 1957; Caloi, Kotsakis & Palombo, 1988).

Conversely, the value of Nei's genetic distance found between Aeolian and Sicilian samples of *P. wagleriana* ($D = 0.147$) falls (1) above that normally encountered for conspecific populations [$D \leq 0.105$ ($I \geq 0.90$); Thorpe, 1983], and (2) into the range obtained from comparisons between well recognized biological species of the genera *Podarcis* (e.g. Nei's $D = 0.141$ between *P. sicula* and *P. muralis*; 24 gene loci scored) (Capula, 1990), and *Lacerta* (e.g. Nei's $D = 0.13$ between *L. oxycephala* and *L. graeca*; 16 gene loci scored) (Mayer & Tiedemann, 1982), revealing marked genetic differentiation between populations. This result is supported by the analyses of *F*-statistics and rates of gene flow, and suggests that the Aeolian and Sicilian populations of the taxon currently regarded as *P. wagleriana* belong to closely related sibling species, which probably diverged from a common ancestor. On the basis of (1) the peculiar geographic distribution, (2) the morphological and chromatic characteristics (see Mertens, 1952, 1955; Arnold & Burton, 1978; Di Palma, 1980), (3) the genetic divergence among populations as indicated by fixed allelic differences, genetic distance data, and fixation index, we suggest the recognition of full specific status for the Aeolian *P. wagleriana*, for which the name *Podarcis raffonei* (Mertens, 1952) (Aeolian wall lizard) is proposed. The present range and proposed formal renaming of the four known subspecies of the Aeolian wall lizard would be as follows: *P. r. raffonei* (Mertens, 1952) (Strombolicchio, 1.6 km NE of Stromboli); *P. r. alvearioi* (Mertens, 1955) (Scoglio Faraglione, 0.3 km W of Salina); *P. r. cucchiarai* Di Palma, 1980 (La Canna, 1.5 km W of Filicudi); *P. r. antoninoi* (Mertens, 1955) (Vulcano) (Fig. 4). The range of *P. wagleriana* would appear therefore to be more limited than previously supposed, being apparently restricted to Sicily, Favignana, Levanzo, Isola Grande dello Stagnone (*P. w. wagleriana*), and Marettimo (*P. w. marettimensis*).

Mechanisms of population differentiation

The Aeolian Archipelago is made up of seven large islands (≥ 3.38 km^2) and several islets (≤ 0.29 km^2) of volcanic origin, which are separated from Sicily by a deep and wide sea channel (sea channel width ranges from 22 km—geographic distance between Vulcano Island and Capo di Milazzo, NE Sicily—to 80 km (geographic distance between Alicudi Island and Capo di Milazzo, NE Sicily)). On the basis of the available geological data, it seems that the Aeolian Islands emerged during the Pleistocene and were always separate from Sicily (Alicudi, Panarea, and portions of Salina and Lipari date back to the Lower–Middle Pleistocene; Vulcano, Stromboli, and the remaining portions of Salina and Lipari date back from the Upper Pleistocene to recent times) (Sacchi, 1961; Ruggieri, 1973; Barberi *et al.*, 1974; Rosi, 1980). However, according to some biogeographic evidence (see e.g. Lanza, 1973; Messina, 1984; Piantelli *et al.*, 1990), it is possible that these volcanic islands began to rise up at the end of the Pliocene, before or contemporaneously with the marine regression that caused

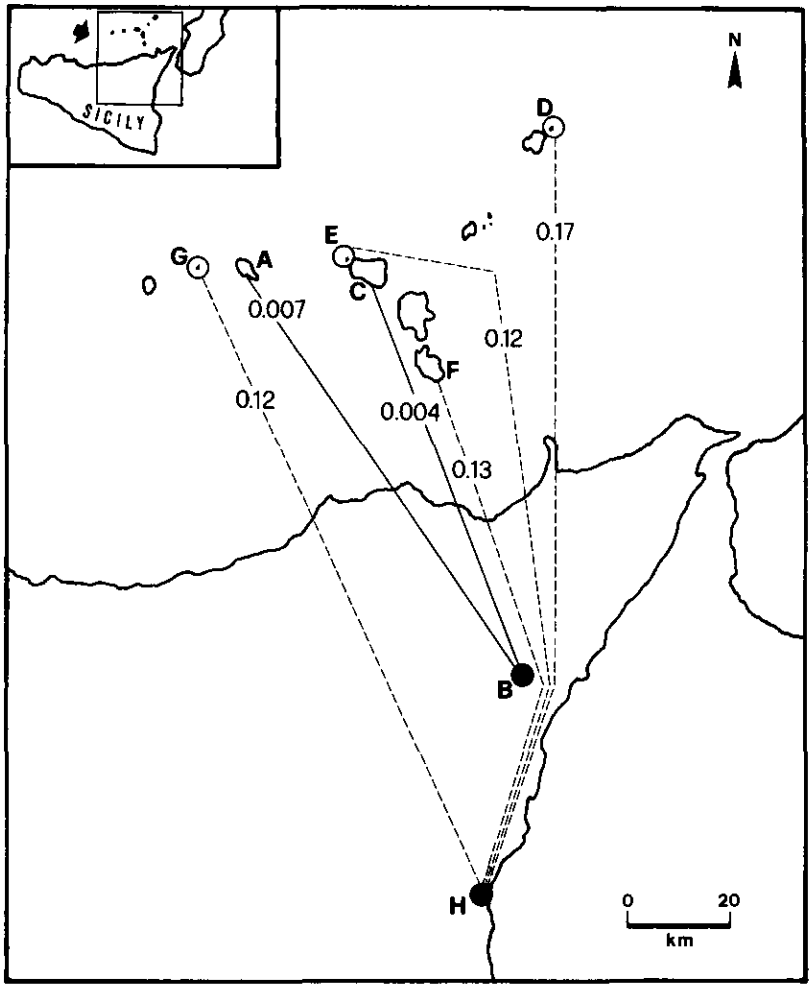

Figure 4. Nei's (1972) standard genetic distances among populations of *Podarcis sicula* (A–C), *P. raffonei* (D–G), and *P. wagleriana* (H) from the Aeolian Islands and NE Sicily. A = Filicudi; B = Linguaglossa; C = Salina; D = Strombolicchio; E = Scoglio Faraglione; F = Vulcano; G = La Canna; H = Primosole. Insert shows location of the study area.

the complete emersion of the continental plate between the Aeolian volcanic arc and Sicily (Furon, 1950; Pasa, 1953). In any case, during the Pleistocene they were probably much larger than at present, and could have been connected, for short periods at least, to Sicily and southern Italian peninsula (Pasa, 1953). Zoological investigations carried out in the Aeolian Archipelago (see e.g. Messina, 1984; Piantelli *et al.*, 1990) would confirm this hypothesis, indicating that the Aeolian fauna is characterized (1) by recent (or very recent) immigrant taxa, i.e. taxa whose occurrence on the islands is best explained by rafting or anthropogenic introduction (e.g. most of the terrestrial malacofauna), and (2) by much more ancient immigrant taxa, i.e. endemic taxa (palaeotyrrhenian elements) which seem to have colonized the Aeolian Islands prior to or during the Pleistocene (e.g. the spider *Harpactea aeoliensis* and the beetle *Ectobius aeoliensis*).

Although the reconstruction of past colonization and evolutionary events is speculative, based on genetic and biogeographic evidence, at least three scenarios can be considered: the colonization process and evolution of *P. raffonei*; the possibility of human introduction of *P. sicula* in the Aeolian Islands; the process of competitive exclusion of *P. raffonei* by *P. sicula*.

The ancestor of *P. raffonei* could have reached the Aeolian area from Sicily during the Roman Regression (Mindel glaciation, Pleistocene), when, according to Pasa (1953), the sea channel between the Aeolian Islands and Sicily closed or was greatly reduced and these insular systems were possibly linked or very close. Due to the subsequent deepening of the sea channel caused both by marine erosion and rising sea level (end of Mindel glaciation), the Aeolian populations remained isolated from the Sicilian ones, and the resulting physical separation could have been responsible for their genetic divergence. This hypothesis is in agreement with the time of evolutionary divergence estimated according to Nei's (1975) formula, which seems to go back to the Middle Pleistocene (0.7 Myr). On the other hand, it must be stressed that the time of geographic isolation estimated by using Sarich's (1977) calibration (corrected by Maxson & Maxson, 1979) would suggest older divergence times, putting back the disjunction events to 2 Myr (Late Pliocene or Early Pleistocene). However in the case of divergence times estimated by using Sarich's (1977) calibration, caution must be used, as (1) this method can produce misleading results due to the fact that genetic distances must be calibrated on the particular 'mix' of fast and slow evolving loci used (Sarich, 1977), and (2) it is now clear that mean rates of amino acid substitution are broadly similar for most proteins and there is no evidence for any sort of bimodality in distribution (see e.g. Skibinski & Ward, 1982; Thorpe, 1989).

The colonization of the Aeolian Islands by *Podarcis sicula* would be much more recent, as indicated by the very low genetic divergence found between the Aeolian and the Sicilian samples of the species. According to Capula *et al.* (1987) and Capula (1990, 1992, 1993), which based their conclusions both on genetic and ecological data, the occurrence of *P. sicula* in the Aeolian Archipelago is probably due to accidental anthropogenic introduction. This is indirectly supported by the evidence that (1) *P. sicula* is a lacertid lizard of broad ecological tolerance (see Nevo *et al.*, 1972), and (2) it is known to have been introduced by man on several Mediterranean islands, e.g. Marettimo, Minorca, Tuscan Archipelago, Adriatic islands (Taddei, 1949; Lanza, 1973; Gorman *et al.*, 1975; Caloi *et al.*, 1988; Corti, Capula & Nascetti, 1989). Based on these data, *P. sicula* would be a faunistic element which has been only recently established on the Aeolian Islands, while *P. raffonei* would be a Pleistocene immigrant (palaeotyrrhenian element), i.e. the native lacertid lizard in the Archipelago, though presently confined to a few islands.

The results of electrophoretic investigations and comparative species distributions in the Aeolian Islands show that the recent invader *P. sicula* must have competed successfully with *P. raffonei*, greatly reducing the range of the latter and probably causing the extinction of most of its populations (Capula *et al.*, 1987; Capula, 1992). As a consequence, the Aeolian wall lizard is presently confined to Vulcano, where it occurs sympatrically with *P. sicula*, and to three tiny islands (Strombolicchio, Scoglio Faraglione, La Canna), where it is the only *Podarcis* species (see Fig. 4). The above mentioned islands can be considered

fringing islands, i.e. small islands that have been probably disconnected by eustatic sea level rise from the adjacent larger island (respectively Stromboli, Salina, Filicudi) within the recent geological past. This indicates that: (1) *P. raffonei* occurred also on Stromboli, Salina and Filicudi; (2) *P. raffonei* has become extinct on the large islands, possibly due to competition with the more opportunistic *P. sicula*; (3) *P. raffonei* was able to survive only on those small islands which were not colonized by *P. sicula*.

According to our observations, each of the tiny islands inhabited by the Aeolian wall lizard supports a small number of lizards. On the other hand, a rough estimate of population size based on a three-year collecting experience at Vulcano—which is the only large Aeolian island (21.2 km^2) at present known to be inhabited by *P. raffonei*—indicates that the species is represented there by a very small number of individuals. On this island, the Aeolian wall lizard appears to have become very rare in historical times, nearly reaching extinction, probably due to hybridization (a high number of F_1 hybrids *P. sicula* × *P. raffonei* were detected on Vulcano by Capula (1993)) and competition with the very abundant *P. sicula* (Capula, 1990, 1992). Documented cases of native lizard species reduced to extinction by the introduction of a reptilian competitor seem to be extremely rare (e.g. Case & Bolger, 1991). However, the case of the Aeolian *Podarcis* certainly can be considered a classic example of competitive exclusion of a native form by a species introduced by man.

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