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Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

Genetic differentiation of populations of Iberian rock-lizards *Iberolacerta (Iberolacerta) sensu* Arribas (1999)

A. P. Almeida, H. D. Rosa, O. S. Paulo and E. G. Crespo

Abstract

The genetic structure and relationships of five populations of the Iberian group of *Iberolacerta* (*sensu* Arribas 1999) were analysed by protein electrophoresis. In this study we confirmed the differentiation between the group of populations from Galicia/Cantabrian/S. Estrela versus the group of populations from de Spanish Central System, Gredos and Guadarrama that are included in the two different species by Arribas (Herpetozoa 9(1/2), 31–56, 1996; Russian J. Herpetol. 6, 1–22, 1999), *Iberolacerta (I.) monticola* and *Iberolacerta (I.) cyreni*, respectively. However, the differentiation level is not high enough to clearly prove their specific discrimination. On the other hand, we did not confirm the subspecific discrimination of the Gredos – *Iberolacerta (I.) cyreni castiliana*, and Guadarrama – *Iberolacerta (I.) cyreni cyreni* populations, proposed by Arribas (1996). These two populations are genetically almost homogeneous. Interestingly, we found an unexpected high genetic similarity between the Galician and the Serra da Estrela populations, presently included into two different subspecies, *Iberolacerta (I.) monticola cantabrica* and *Iberolacerta (I.) monticola monticola*, respectively. Their genetic similarity is even higher than that between the populations of Galicia and the Cantabrian Mountains, which are both included in the same subspecies, *cantabrica*. This result suggests that the populations of Galicia and Serra da Estrela would have maintained contacts, possibly through the north of Portugal, until relatively recent times. Their separation is thus probably post-glacial. Some evidence also points to the existence of relatively recent contacts between the population of Serra da Estrela and those of the Central System, particularly, with the neighbouring Peña de Francia population. With the cautions imposed by the reduced sample size of our analysis, the significant differentiation of the populations from Galicia and the Cantabrian Mountains allow us to suggest that this last population may not be the result of a recent expansion of the Galician population as Arribas (1996) suggests, but, more likely, the result of a fragmentation process of a more ancient and wider north-eastern distribution area of this group of rock-lizards.

Key words: Rock-lizards – genetic differentiation – allozymes

Introduction

Until a few years ago the rock-lizard populations endemic to the Iberian Peninsula were considered to belong to one species, *Lacerta monticola* Boulenger 1905, distributed over several widely dispersed regions of the northern half of the Iberian Peninsula (Barbadillo 1987). According to this single-species view a subspecies *L. m. cantabrica* Mertens 1929 would occur in a area of the Cantabrian Mountains and Galicia (NW of Spain), ranging from the sea level on the northern coastal edge of Galicia up to 1700 m altitude in the mountains. The subspecies *L. m. cyreni* Müller and Hellmich 1937, structured into several subpopulations would be found in the region of the Spanish Central System Mountains and limited to altitudes over 1500 m in the sierras of Peña de Francia, Gredos and Guadarrama, with no actual contact to each other. In the Westernmost part of this System, in the Central plateau of the Serra da Estrela (Portugal), the subspecies *L. m. monticola* Boulenger 1905 would occur, restricted to altitudes over 1500 m. Finally, a fourth subspecies, *L. m. bonnali* Lantz 1927, would inhabit the Pyrenees.

The fragmentation of the distribution area of this species was thought to be a consequence of a post-glacial fragmentation of a previous wider distribution range due to progressive warming and increased aridness of the environment (Pérez-Mellado et al. 1993), and possibly also to the competition with small lizards of the genus *Podarcis* (Pérez-Mellado 1997). A distinct species status, *L. bonnali* (Arribas 1993a; Pérez-Mellado et al. 1993), of the Pyrenean populations, was subsequently recognized. Later it was verified that the populations of this new species constitute a heterogeneous group in which at least three distinct taxa could be discriminated, *Lacerta bonnali*, *Lacerta aurelioi* and *Lacerta aranica*, probably differentiated from each other during the Pleistocene period (Arribas 1993a, b, 1994; Odierna et al. 1996a, b; Mayer and Arribas 1996).

If this is the case, the actual populations of *L. monticola* would be restricted to the Cantabrian Mountains and Galicia (ssp. *cantabrica*), Serra da Estrela (ssp. *monticola*) and the Spanish Central System mountain (ssp. *cyreni*). However, in the case of the last subspecies, it was noticed, in particular with respect to the Guadarrama population, that this population has some morphological and karyological features that seem to differentiate it from the populations of the other subspecies (Brown and Pérez-Mellado 1993; Arribas 1993a; Odierna et al. 1996a, b). Although the Cantabrian/Galician (ssp. *cantabrica*) and Estrela (ssp. *monticola*) populations are morphologically distinct, when compared with those from the Spanish Central System (ssp. *cyreni*), they appear similar to each other, not only in their pholidotic, osteological and coloration/colour patterns (Pérez-Mellado et al. 1993), but also in their allozymatic (Mayer and Arribas 1996) and karyological (Odierna et al. 1996a, b) traits. The *cantabrica* and *monticola* populations display the same karyotype containing 36 acrocentric chromosomes, whereas *cyreni*, even though it has the same chromosome number, differs from them in the localization of the nucleolar organizer (NOR) and in the banding pattern and heterochromatin content of the sexual chromosome (W).

On the basis of what was previously suggested, Arribas (1996) performed an extensive phenetic analysis (including biometric, pholidotic and coloration/colour characters) of these populations. He explicitly proposed the attribution of a discriminate specific status to the populations of the Spanish Central System – *L. cyreni*. He also ascribed different subspecific levels to the populations of Guadarrama

(*L. c. cyreni*), Gredos (*L. c. castiliana*) and Peña de Francia (*L. c. martinezricai*).

According to this author, the ancestor of *L. monticola* and *L. cyreni* would have originated from the north-western part of the Iberian Peninsula – Galicia and adjacent regions. This ancestor would have extended over a large part of the north of Portugal and the Spanish Central System and, during an interglacial Pleistocene period, would have been split into two main branches. A southern one, in the cool and humid refuge of the Central System Mountains, giving rise to *L. cyreni*. The other, in the north-west of the Iberian Peninsula, giving rise to *L. monticola*. After the end of Würm glacial period the general rise in temperature could have caused the present isolation between the various actual *L. cyreni* populations. He also noticed that the population of Peña de Francia, although included in *L. cyreni*, shares some characters common with *L. monticola*, that, in his opinion, could be explained as being a reminiscence of common ancestral characters. Recently, in a cladistic study on the phylogeny and relationships of the mountain lizards of Europe and Near East (*Archaeolacerta* Mertens 1921, *sensu lato*) and their relationships in the context of the Euroasian lacertid radiation, Arribas (1999) proposed a new taxonomic re-arrangment of this group, placing the Iberian and Pyrenean forms in a new genus *Iberolacerta*. The populations of *L. monticola* from Galicia/Cantabrian Mountains (ssp. *cantabrica*) and Serra da Estrela (ssp. *monticola*), together with *L. horvathi* (Eastern Alps and North of Dinaric Chains), were put to the subgenus *Iberolacerta s*.str., those from Pyrenees (*L. bonnali*, *L. aranica* and *L. aurelioi*) to the subgenus *Pyrenesaura*.

To evaluate the present conservation status and future viability of the *I. monticola* (*sensu* Arribas 1999) population from Serra da Estrela (Portugal), we performed an analysis of this population considering mainly demographic aspects (Moreira et al. 1996, unpublished data). An evaluation of the genetic structure, variability and degree of differentiation of this population compared with other genetically related populations was also studied to define its conservation status and viability more clearly.

Considering the deep taxonomic reorganization to which this group of rock-lizards has been subjected, and the fact that its genetic differentiation is far from being well understood (previous studies are based on a small sample size – Mayer and Arribas 1996), we present here the results obtained in our genetic study which may contribute to the clarification of the problem.

Materials and methods

Samples were collected from populations throughout the distribution area of the Iberian *Iberolacerta* group (*sensu* Arribas 1999): Galicia and Cantabrian mountains, *Iberolacerta* (*I.) monticola cantabrica*, *Serra da Estrela*, *Iberolacerta* (*I.) monticola monticola*, and Spanish Central System – Gredos, *Iberolacerta* (*I.) cyreni castiliana* and Guadarrama, *Iberolacerta* (*I.) cyreni cyreni* (Fig. 1). A sample of *Podarcis muralis* from the Cantabrian Mountains was also used as a reference group for the genetic study.

Using horizontal starch gel and cellulose acetate electrophoresis, a total of 20 presumptive loci were scored, coding for 14 enzymes and two blood proteins. The protein systems studied, as well as the respective electrophoretic conditions and tissues used are presented in Table 1. The *loci* and the alleles were assigned according to Pasteur et al. (1987).

Data analysis was performed using the software BIOSYS-1 from Swofford and Selander (1981) and GENEPOP from Raymond and Rousset (1995).

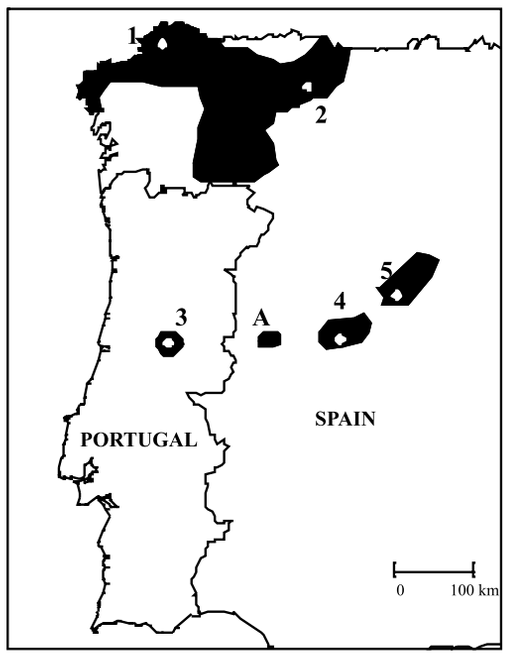

Fig. 1. Distribution area of the Iberian group of rock-lizards, *Iberolacerta (I.) monticola* and *Iberolacerta (I.) cyreni* (*sensu* Arribas 1999). 1, Galicia, *Iberolacerta (I.) monticola cantabrica*; 2, Cantabrian mountains, *Iberolacerta (I.) monticola cantabrica*; 3, Serra da Estrela, *Iberolacerta (I.) monticola monticola*; 4, Sierra de Gredos, *Iberolacerta (I.) cyreni castiliana*; 5, Sierra de Guadarrama, *Iberolacerta (I.) cyreni cyreni*; A, Sierra Peña de Francia, *Iberolacerta (I.) cyreni martinezricai*

For each sample, the genotype frequencies observed were compared to those expected (Hardy–Weinberg equilibrium) and the differences tested with the chi-square test with Yates's correction for continuity, and using pooled genotypes if more than two alleles were present.

Genetic variability was quantified from the average heterozygosity observed, calculated and corrected (Nei 1978), from the percentage of polymorphic *loci* (95 and 99% criteria) and from the number of alleles per *locus*. The genetic heterogeneity among samples was evaluated with chi-square tests in contingency tables, grouping the populations under study at subspecific and specific levels and in the totality of the populations.

The genetic differentiation between the populations was estimated through genetic distance indexes (Nei 1978) supplied by BIOSYS–1 and by F statistics from Weir and Cockerham (1984) obtained by the program DIPLOIDL (Weir 1990). Summaries of genetic distance (Nei 1978) were generated by UPGMA clustering (Sneath & Sokal l973).

The eventual occurrence of isolation by distance was found by comparing the Nei (1978) genetic distance matrices with the geographic distance between population pairs, using a Mantel randomization test (Briers 1999) and the Slatkin (1993) graphic method carried out using the software DIST (Slatkin). The values of gene flow among populations was estimated using the M Slatkin (1993) index $[M = (1/F_{ST} - 1)/4]$, with $F_{ST} = \theta$, according to Cockerham and Weir (1993).

Results

Of the 20 loci successfully examined, 10 proved to be monomorphic and common to all *Iberolacerta* populations studied (*Adh*, *Ak*, *Fum*, *Gld*, *Gpi*, *Ldh-1*, *Mdh-1*, *Mdh-2*, *Pep-1*, *Hb*). The remaining 10 loci were represented by two or three alleles (Table 2). A group of eight alleles (Ak^{90}, Gpi^{70}, $Ldh\text{-}1^{150}$, $Mdh\text{-}2^{200}$, $Pep\text{-}1^{110}$, $Pgm\text{-}1^{80}$, Sod^{60}, Hb^{125}) distinguish *P. muralis* from all the *Iberolacerta* populations.

Table 1. Protein loci scored, electrophoresis media, tissue sources and buffer systems used

Proteins	E. no.	*Locus*	Electrophoresis	Tissue	Buffer
Alcohol dehydrogenase (ADH)	E.C.1.1.1.1	*Adh*	A	L	I
Adenylate kinase (AK)	E.C.2.7.4.3	*Ak*	B	L	II
Aspartate aminotransferase (AAT)	E.C.2.6.1.1	*Aat-1,2*	B	H,M	II
Albumin (ALB)	–	*Alb*	D	Hm	IV
Malic enzyme (ME)	E.C.1.1.1.40	*Me-1*	A	H,M	I
Esterase (EST)	E.C.3.1.1	*Est-1,2*	B	L	III
Phosphoglucomutase (PGM)	E.C.2.7.5.1	*Pgm-1*	B	L	II
Fumarase (FUM)	E.C.4.2.1.2	*Fum*	A	L	I
Glucose-6-phosphate isomerase (GPI)	E.C.5.3.1.9	*Gpi*	B	L	III
Glutamate dehydrogenase (GLD)	E.C.1.4.1.2	*Gld*	B	L	II
Haemoglobin (HB)	–	*Hb*	D	Hm	IV
l-Lactate dehydrogenase (LDH)	E.C.1.1.1.27	*Ldh-1,2*	B	H,M	II
Malate dehydrogenase (MDH)	E.C.1.1.1.37	*Mdh-1,2*	B	H,M	II
Mannose-6-phosphate isomerase (MPI)	E.C.5.3.1.8	*Mpi*	B	L	II
Peptidase leucil-alaninic (PEP)	E.C.3.4.11	*Pep-1*	B	L	III
Superoxide dismutase (SOD)	E.C.1.15.1.1	*Sod*	B	L	V

H, heart; L, liver; Hm, haemolysate; M, muscle.
Electrophoresis: A, on acetate cellulose gel (Hebert and Beaton 1989); B, on starch gel (Pasteur et al. 1987); D, on acetate cellulose strips (Gelman 1970).
Buffer: I, Tris-Glycine pH 8.5 (Hebert and Beaton 1989); II, Tris-Citrate pH 8.0 (Pasteur et al. 1987); III, LiOH pH 8.3 (Pasteur et al. 1987); IV, Tris-Barbital pH 8.8 (Gelman 1970); V, Tris-Citrate pH 6.7/6.3 (Pasteur et al. 1987).

The loci *Aat-2*, *Ldh-2*, *Pgm-1* and *Sod* gave very little information about the differentiation of the populations within the *Iberolacerta* group, revealing different rare alleles in each of the various populations. In this regard the most informative loci were *Alb*, *Aat-1*, *Est-1*, *Est-2*, *Me-1* and *Mpi* (Table 2).

Within-population, the *Alb* locus was monomorphic or very slightly polymorphic revealing an Alb^{100} allele that was unique and common to the populations of Serra da Estrela and Galicia/Cantabrian. It likewise occurred, but at a very low frequency, in Gredos. The Guadarrama population showed a unique Alb^{105} allele which was the predominant one in the Gredos population. With respect to *Aat-1*, the Galician and Cantabrian populations showed only the $Aat\text{-}1^{100}$ allele which was also highly predominant in the population of Serra da Estrela. $Aat\text{-}1^{100}$ and $Aat\text{-}1^{50}$ were found to coexist in high frequencies in the Gredos and Guadarrama population, $Aat\text{-}1^{50}$ being rare in the Serra da Estrela.

Regarding Me-1, the Me-1^{130} allele was highly predominant in the Galician, Cantabrian and Serra da Estrela populations; in contrast, the Me-1^{100} allele prevailed in the Gredos population.

Concerning the *Mpi* and *Est-2* loci, the Gredos and Guadarrama populations possessed only one allele, Mpi^{100} and $Est\text{-}2^{100}$, respectively. The Mpi^{100} was also present, although with lower frequencies, in the Serra da Estrela and Cantabrian Mountains populations, but was absent in Galicia; the $Est\text{-}2^{100}$ allele was also present in the Serra da Estrela and Galicia populations, but absent in the Cantabrian population.

Est-1 was found to be monomorphic for $Est\text{-}1^{100}$ in the Serra da Estrela population. The allele $Est\text{-}1^{90}$, predominated in Galicia but showed only low frequencies in the Cantabrian Mountains and in the Gredos and Guadarrama populations.

Discriminating alleles existed in all populations in at least one locus, although always rare or with a low frequency, e.g. $Aat\text{-}1^{160}$ and $Pgm\text{-}1^{115}$ from Serra da Estrela, $Aat\text{-}2^{55}$ and $Ldh\text{-}2^{130}$ from Galicia, Mpi^{155} from the Cantabrian Mountains, Alb^{110} and $Me\text{-}1^{80}$ from Gredos and Sod^{140} from Guadarrama.

The populations of Galicia and of the Cantabrian Mountains, as a whole, did not share any exclusive alleles but the populations of Gredos and Guadarrama had one private allele, Alb^{105}. The populations of Serra da Estrela and those of the Central System shared the $Aat\text{-}1^{50}$ allele, which was not present in the Galician and Cantabrian populations.

Hardy–Weinberg equilibrium was verified for all the loci in the various populations studied. Using the contingency chi-square tables for the polymorphic loci, we can conclude in general that the populations studied are not a panmictic unity. The *Alb*, *Aat-1*, *Est-1*, *Est-2*, *Me*, and *Mpi* loci showed the most significant heterogeneity, $p < 0.001$. The heterogeneity of the Galician and Cantabrian populations altogether, turned out to be highly significant ($p < 0.001$), mainly due to the information from the *Est-1*, *Est-2* and *Mpi* loci. In contrast, the Gredos and Guadarrama populations, with $p = 0.048$, were very close to the threshold of acceptance of the null hypothesis, or, in other words, to panmixis.

The parameters of genetic variability for all the sampled populations are presented in Table 3. The Serra da Estrela population is the one which revealed the highest polymorphism and heterozygosity values. The Cantabrian Mountain population, in the contrary, showed the lowest values which can partly be explained by the rather small number of individuals analysed. It is therefore interesting mention that the *P. muralis* population of the same region shows also very low levels of polymorphism and heterozygosity, revealing variability in only one of the loci studied (*Hb*).

Concerning the populations of the Spanish Central System, Gredos showed higher variability as is evident from the greater number of allele per locus, greater percentage of polymorphic loci and the higher level of heterozygosity, compared with Guadarrama, where this variability was very low. The Galicia population showed intermediate values of polymorphism and heterozygosity in comparison with those from the Serra da Estrela and the Spanish Central System.

The genetic distances between populations of the Iberian *Iberolacerta* group (and *P. muralis*) are presented in Table 4. Concerning the *Iberolacerta* populations the highest average genetic distances were found between the two Central System

Table 2. Allele frequencies for the polymorphic loci scored in *Iberolacerta* group

		I. (I.) monticola			*I. (I.) cyreni*		
		monticola	*cantabrica*		*castiliana*	*cyreni*	*Podarcis muralis*
Locus	*alleles*	Estrela	Galicia	Cantabrian	Gredos	Guadarrama	Cantabrian
Aat-1							
	n	21	17	11	21	23	10
	160	0.024	–	–	–	–	–
	100	0.952	1.000	1.000	0.476	0.696	1.000
	50	0.024	–	–	0.524	0.304	–
Aat-2							
	n	19	11	8	21	10	7
	100	1.000	0.864	1.000	1.000	1.000	–
	55	–	0.136	–	–	–	1.000
Alb							
	n	24	15	13	21	24	13
	110	–	–	–	0.024	–	–
	105	–	–	–	0.952	1.000	–
	100	1.000	1.000	1.000	0.024	–	1.000
Est-1							
	n	15	17	9	8	14	5
	100	–	0.118	1.000	0.813	0.786	–
	90	1.000	0.882	–	0.188	0.214	1.000
Est-2							
	n	10	10	7	9	15	10
	100	0.450	0.700	–	1.000	1.000	–
	50	0.550	0.300	1.000	–	–	1.000
Ldh-2							
	n	21	17	13	21	23	12
	130	–	0.029	–	–	–	–
	100	1.000	0.971	1.000	1.000	1.000	1.000
Me-1							
	n	22	15	12	21	22	12
	130	0.841	0.867	1.000	–	0.023	–
	100	0.159	0.133	–	0.881	0.977	1.000
	80	–	–	–	0.119	–	–
Mpi							
	n	24	17	13	21	21	11
	155	–	–	0.038	–	–	1.000
	115	0.708	1.000	0.385	–	–	–
	100	0.292	–	0.577	1.000	1.000	–
Pgm-1							
	n	24	17	13	21	21	11
	115	0.083	–	–	–	–	–
	100	0.917	1.000	1.000	1.000	1.000	–
	80	–	–	–	–	–	1.000
Sod							
	n	22	16	11	19	19	11
	140	–	–	–	–	0.026	–
	100	1.000	1.000	1.000	1.000	0.974	–
	60	–	–	–	–	–	1.000

n, number of individuals sampled for each locus.

populations (Gredos and Guadarrama) in comparison with the northern populations from Galicia and the Cantabrian Mountains ($\overline{D} = 0.191$, ranging from 0.184 to 0.200), followed by those between the same two populations from the Central System and the population from Serra da Estrela ($\overline{D} = 0.185$, ranging from 0.182 to 0.189). The average genetic distance between population found among all *Iberolacerta* populations was $\overline{D} = 0.130$, ranging from 0.002 (Gredos/Guadarrama) to 0.200 (Galicia/Gredos).

As expected, higher distances were found between the populations of *Iberolacerta* and the species used as reference, *P. muralis* ($\overline{D} = 0.842$, ranging from 0.718 to 0.982).

These relationships may be easily visualised in the distances dendrogram (UPGMA) of Fig. 2. The Serra da Estrela, Galicia and Cantabrian Mountain group, *Iberolacerta (I.) monticola* (*sensu* Arribas 1999) is clearly separated from the Gredos and Guadarrama group, *Iberolacerta (I.) cyreni* (*sensu* Arribas 1999). The genetically closer populations are the pairs Serra da

Table 3. Measures of genetic diversity in the Iberian *Iberolacerta* group (and *P. muralis*) populations

	I. (I.) monticola			*I. (I.) cyreni*		
	monticola	*cantabrica*		*castiliana*	*cyreni*	*P. muralis*
	Estrela	Galicia	Cantabrian	Gredos	Guadarrama	Cantabrian
N	19.9 (1.2)	14.4 (1.0)	11.4 (0.5)	17.6 (1.2)	19.4 (1.2)	10.3 (0.5)
A	1.3 (0.1)	1.3 (0.1)	1.1 (0.1)	1.3 (0.1)	1.2 (0.1)	1.0 (0.1)
$P_{95\%}$	20.0	20.0	5.0	15.0	10.0	5.0
$P_{99\%}$	25.0	25.0	5.0	20.0	20.0	5.0
Mean heterozygosity	0.085 (0.042)	0.066 (0.034)	0.023 (0.023)	0.047 (0.029)	0.029 (0.018)	0.015 (0.015)
H–W expected	0.073 (0.034)	0.060 (0.028)	0.027 (0.027)	0.057 (0.031)	0.044 (0.027)	0.025 (0.025)

N, mean no. of individuals sampled; A, mean no. of alleles per locus; $P_{95\%}$ and $P_{99\%}$, percentage of polymorphic loci (95% and 99% criteria). Standard errors are in parentheses.
H–W, Hardy–Weinberg.

Table 4. Genetic distances: D (Nei 1978) between the Iberian *Iberolacerta* (and *P. muralis*) populations

	Iberolacerta (I) monticola			*Iberolacerta (I) cyreni*		
	monticola	*cantabrica*		*castiliana*	*cyreni*	*P. muralis*
	Estrela	Galicia	Cantabrian	Gredos	Guadarrama	Cantabrian
Iberolacerta (I.) monticola						
monticola/Estrela	–	0.008	0.070	0.189	0.182	0.718
cantabrica/Galicia		–	0.089	0.200	0.193	0.751
/Cantabrian			–	0.187	0.184	0.812
Iberolacerta (I.) cyreni						
castiliana/Gredos				–	0.002	0.982
cyreni/Guadarrama					–	0.946
P. muralis/Cantabrian						–

Estrela/Galicia and Gredos/Guadarrama. The Cantabrian population is closest to the first pair although somewhat removed from it.

The *F*-statistics (Weir and Cockerham 1984) for the Iberian *Iberolacerta* populations are summarised in Table 5. The weak positive average value of f indicates that there is a slight excess of homozygotes within populations. The strongly positive value of *Est-1* is prominent. The average values of coancestry indexes, θ, are particularly high ($\theta = 0.680$), due to the differentiation in the *Aat-1*, *Alb*, *Est-1*, *Est-2*, *Me-1* and *Mpi* loci, indicating the existence of a high differentiation between populations.

The values of gene flow between the populations are presented in Table 6 together with the respective geographic distances.

Values of $M < 1$ are recorded for almost all the pairs, which indicates a lack of gene flow. Only the Serra de Estrela/Galicia ($M = 2.141$) and the Gredos//Guadarrama ($M = 6.023$) pairs, show values of $M > 1$.

The relationships between the genetic and the geographic distances, evaluated by the Mantel randomization test, demonstrated that independence exists between the levels of genetic differentiation of the populations and their geographic distances ($r = 0.663$, $p > 0.05$). Furthermore, the application of the

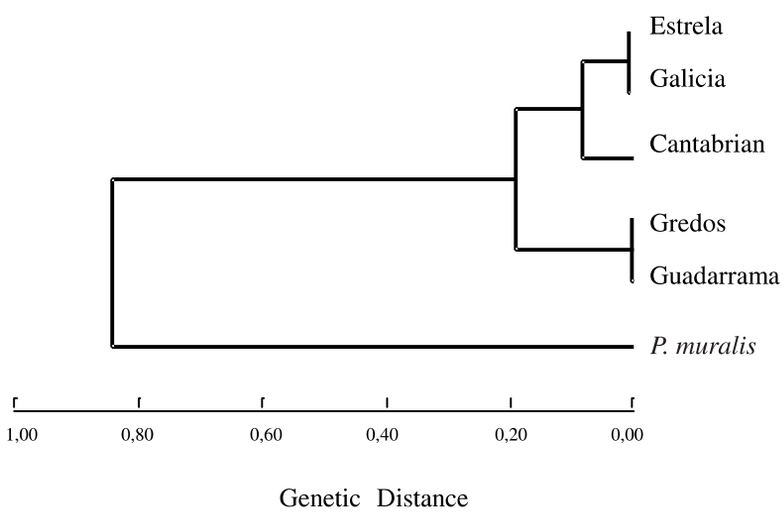

Fig. 2. UPGMA dendrogram based on genetic distances, D (Nei 1978), between the five populations of the Iberian *Iberolacerta* group and the Cantabrian population of *P. muralis* used as a reference

Table 5. *F* statistics indexes of Weir and Cockerham (1984) estimate for each polymorphic locus for all populations of the Iberian *Iberolacerta* group

Locus	f	*F*	θ
Aat-1	0.034	0.326	0.302
Aat-2	−0.121	0.011	0.118
Alb	−0.011	0.967	0.968
Est-1	0.561	0.864	0.691
Est-2	−0.377	0.479	0.622
Ldh-2	−0.003	0.001	0.004
Me-1	0.010	0.738	0.736
Mpi	0.066	0.708	0.689
Pgm-1	−0.064	−0.005	0.056
Sod	0.003	−0.001	0.003
Mean	0.043	0.694	0.680

graphic test (Slatkin 1993) that relates the M decimal logarithms with the respective geographic distances decimal logarithms, points to the absence of isolation due to distance. This relation is weakly linear ($\log_{10}$ M = 4.7353 – 2.2204 $\log_{10}$ d) with a low regression coefficient ($R^2 = 0.298$), which also demonstrates that isolation by distance does not explain the different levels of genetic differentiation between these populations.

Discussion

Genetic diversity of reptiles evaluated through electrophoretic analysis of proteins/enzymes is very variable. The mean values of heterozygosity for reptiles reported by Nevo et al. (1984) in 70 species and by Ward et al. (1992) in another 85 species, are $\overline{H} = 0.055$ and $\overline{H} = 0.078$, respectively. The mean value of H we found in the populations of the Iberian group of *Iberolacerta* was slightly lower $\overline{H} = 0.050$ (ranging from 0.023 to 0.085). At the within-population level, the rock-lizards of Serra da Estrela show the highest genetic diversity expressed by the values of heterozygosity, number of alleles per locus and proportion of polymorphic loci. Conversely, the population of Guadarrama, and particularly the population of the Cantabrian Mountains, show a highly reduced variability. However, in the latter case, the low values might be due to the very small number of individuals analysed. Within populations, the endogamic processes are not relevant in the majority of the populations, as is indicated by the weakly positive values of f (Table 5).

Our results reveal the existence of a remarkable genetic differentiation between populations. This was predictable taking into account the fragmentation of the distribution area of these lizards, resulting in geographic isolation for many of them. This isolation is confirmed not only by the presence of unique, although rare alleles, in all the populations, but also by the high values of θ (= 0.680) and values of $M < 1$, indicating reduced or null gene flow among almost all the populations.

In agreement with information that has already been obtained by employing morphological, allozymatic (Arribas 1996; Mayer and Arribas 1996) and karyological criteria (Odierna et al. 1996a, b), we were able to confirm the accentuated genetic differentiation between the populations from Galicia, Cantabrian Mountains and Serra da Estrela in comparison with the populations from the Spanish Central System mountains (Gredos and Guadarrama); the former populations included in *Iberolacerta (I.) monticola*, and the latter in *Iberolacerta (I.) cyreni* (*sensu* Arribas 1999). However, the values of genetic distance that were found between these two sets of populations (ranging from 0.182 to 0.200) are not high enough to suggest that they are two well-discriminate species.

One the other hand, our results do not support the discriminate subspecific status of the Gredos (subspecies *castiliana*, *sensu* Arribas 1999) and Guadarrama (subspecies *cyreni*, *sensu* Arribas 1999) populations of *Iberolacerta (I.) cyreni*, as these populations are genetically very close (D = 0.002).

Surprisingly we found a considerable genetic differentiation between the Galician and Cantabrian populations (D = 0.089), both currently included in the subspecies *cantabrica* of *Iberolacerta (I.) monticola* and an unexpected high affinity between the populations from Galicia and Serra da Estrela (D = 0.008), which are included in the *I. monticola* subspecies *cantabrica* and *monticola*, respectively. This affinity is even higher than the one that exists between the two populations of *cantabrica* analysed. (Table 4, Fig. 2).

In terms of the paleogeographic evolution of this group our results suggest that the coastal NW peninsular populations have maintained close contacts, through the north of Portugal, until relatively recent times. Considering their close genetic similarities, it is probable that the separation of the Galician population nucleus from the Serra da Estrela nucleus occurred only in post-glacial times.

We also suggest that the high genetic variability of the Serra da Estrela population, which is nowadays geographically well isolated in a restricted area of only 55 km^2, could be due to the relatively high number of individuals, estimated at about 600.000 individuals (Moreira et al. 1996, unpublished data), but it could also be due to its intermediate geographical

Table 6. Gene flow estimates M (above diagonal) and geographic distance/kms (below diagonal), between pairs of populations of the Iberian *Iberolacerta* group. M = $(1/\theta - 1)/4$ (Slatkin 1993; Cockerham and Weir 1993)

	Iberolacerta (I.) monticola			*Iberolacerta (I.) cyreni*	
	monticola	*cantabrica*		*castiliana*	*cyreni*
	Estrela	Galicia	Cantabrian	Gredos	Guadarrama
Iberolacerta (I.) monticola					
monticola/Estrela	–	2.141	0.219	0.101	0.092
cantabrica/Galicia	340	–	0.139	0.084	0.075
/Cantabrian	356	212	–	0.069	0.058
Iberolacerta (I.) cyreni					
castiliana/Gredos	204	412	300	–	6.023
cyreni/Guadarrama	320	446	282	120	–

position between the northern and eastern populations of this Iberian group of *Iberolacerta* (Fig. 1). In addition to the contacts that it could have maintained until recent times with the northern populations, there are several pieces of evidence pointing to the possibility that it could also have had relatively recent contacts with the Spanish Central System population of the Sierra de Peña de Francia. In fact, it shows some morphological features in common with this last population, which, although not included in our study, is geographically, the closest population in the Spanish Central System and is included in the *Iberolacerta (I.) cyreni* with the status of a discriminate subspecies – *martinezricai* (Arribas 1996). In addition, the Serra da Estrela population shares a rare allele (*Aat-1*^{50}) with the Central System populations, which is not present in the Northern Galician and Cantabrian populations.

Finally, the considerable genetic differentiation between the populations from Galicia and the Cantabrian Mountains, allow us to suggest that this latter population may not be the result of a recent expansion of the populations from Galicia (Arribas 1996) but, more probably, the consequence of a fragmentation process of a more ancient and wider north-eastern distribution area of this Iberian group of rock-lizards.

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Zusammenfassung

Genetische Differenzierung der Populationen der Iberischen Felseidechsen

Die genetische Struktur und die Verwandtschaftsbeziehungen zwischen fünf Populationen der iberischen Gruppe von Iberolacerta (*sensu* Arribas 1999) wurden mit Hilfe der Protein-Elektrophorese untersucht. Wir bestätigen durch diese Analyse die Abtrennung der Populationsgruppen von Galizien/Cantabrien/S. Estrela einerseits und den Populationsgruppen aus dem spanischen Zentral System, Gredos und Guadarrama andererseits, in die zwei verschiedenen Arten *Iberolacerta (I.) monticola* und *Iberolacerta (I.) cyreni* (Arribas, Herpetozoa 9(1/2), 31–56, 1996; Russian J. Herpetol. 6, 1–22, 1999). Allerdings erscheint das Ausmaß der Differenzierung nicht groß genug, um den eindeutigen Status als Arten zu beweisen. Hingegen konnten wir die Unterscheidung der Populationen von Gredos als Unterart *Iberolacerta (I.) cyreni castiliana* und von Guadarrama als Unterart *Iberolacerta (I.) cyreni cyreni* nicht bestätigen. Die beiden Populationen sind genetisch fast homogen. Interessanterweise fanden wir eine unerwartet starke genetische Ähnlichkeit zwischen den Populationen von Galizien und von Serra de Estrela, die derzeit den beiden verschiedenen Unterarten *Iberolacerta (I.) monticola cantabria* bzw. *Iberolacerta (I.) monticola monticula* zugeordnet werden. Ihre genetische Ähnlichkeit ist sogar größer als die zwischen den Populationen der Galizischen und Cantabrischen Berge, die der gleichen Art, *I. cantabrica*, zugeordnet werden. Dies läßt vermuten, daß die Populationen von Galizien und Serra da Estrela bis vor relativ kurzer Zeit in Kontakt standen, vermutlich über den Norden Portugals. Ihre Trennung erfolgte wahrscheinlich nach der Eiszeit. Einige Daten weisen auch darauf hin, daß bis vor relativ kurzer Zeit zwischen der Population von Serra da Estrela und denen des Zentralsystems, besonders mit der benachbarten Population von Peña de Francia Kontakt bestanden haben muß. Mit einiger Vorsicht, die bei der reduzierten Stichprobengröße unserer Analyse angebracht ist, erlaubt uns die signifkante Differenzierung zwischen den Populationen der Galizischen und der Cantabrischen Berge anzunehmen, daß letztere Population nicht auf eine rezente Expansion der galizischen Population zurückgeht, wie dies Arribas (1996) angenommen hat, sondern das Ergebnis eines Fragmentierungsprozesses in einem älteren und weiter nordöstlicheren Verbreitungsgebiet dieser Gruppe der Felseidechsen ist.

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Authors' addresses: Eduardo G. Crespo (for correspondence), Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, bloco C2 piso 3°, Rua Ernesto de Vasconcelos, Campo Grande, P-1749–016 Lisboa, Portugal. E-mail: egcrespo@fc µl.pt) A. P. Almeida, H. D. Rosa and O. S. Paulo, Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, bloco C2 piso 3°, Rua Ernesto de Vasconcelos, Campo Grande, P-1749–016 Lisboa, Portugal.