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MAJOR PATTERNS OF POPULATION DIFFERENTIATION IN THE IBERIAN SCHREIBER'S GREEN LIZARD (*LACERTA SCHREIBERI*) INFERRED FROM PROTEIN POLYMORPHISM

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The genetic characteristics of the Iberian Schreiber's green lizard (*Lacerta schreiberi*) remain largely unknown. We investigated the population structure of this species using conventional electrophoresis and isoelectric focusing to screen 24 protein loci from 11 representative populations of the Iberian Peninsula. Thirteen polymorphic loci displaying a total of 30 alleles revealed significant partitioning of genetic variation among populations (F_{ST}=0.448). Analysis of standard genetic variability measures and allelic distribution profiles indicated that the most variable populations are located in the main distribution area of the species: the north-western corner of the Iberian Peninsula and the Spanish Central System. In contrast, southern isolated populations showed depleted levels of genetic diversity, indicating that severe restrictions to gene flow together with small population sizes are promoting genetic uniformity. We suggest that present-day patterns of genetic diversity in *L. schreiberi* populations are concordant with the biogeographical hypothesis of a recent expansion to the south followed by a history of contraction and fragmentation resulting in today's isolated southern populations.

Key words: electrophoresis, isoelectric focusing, Lacertidae, population genetics

INTRODUCTION

Schreiber's green lizard, *Lacerta schreiberi* (Bedriaga, 1878) is endemic to the Iberian Peninsula. Its distribution is generally continuous in north-western Iberia down to the Tejo river, with several isolated populations occurring in central and southern regions such as the mountains of Sintra, Monchique, Cercal and S. Mamede in Portugal, and Las Villuercas, Guadalupe, Toledo and Morena (San Andrés) in Spain (Marco & Pollo, 1993; Barbadillo *et al.*, 1997; Brito *et al.*, 1996) (Fig. 1). *L. schreiberi* is a medium-sized lizard (maximum adult snout-vent length of 125 mm (Galán, 1984)), inhabiting stream and river margins, in areas characterised by the rainy winters and mild summers typical for the Atlantic climate. The isolated southern populations are restricted to regions that constitute "Atlantic islands" surrounded by areas strongly influenced by Mediterranean climate.

Based on biogeographical data, Salvador (1974) suggested that *L. schreiberi* originated in north-west Iberia after splitting from the common ancestor of the green lizard group. Lutz & Mayer (1985) provided an estimate of this event as 3 to 4 million years ago, based on an immunological albumin clock. De la Riva (1987) followed Salvador (1974), adding that the species may have dispersed to the south and to the east from a north-western speciation centre, as a consequence of Pleistocenic climatic fluctuations. Later, Marco & Pollo (1993) further suggested that *L. schreiberi* might once have been distributed throughout the Iberian Peninsula. In this case, central and southern isolated populations represent the remnants of that wider distribution range that are now restricted to mountains where environmental conditions are still suitable.

Allozyme electrophoresis has been extensively used to investigate the genetic structure of natural populations in a wide range of plant and animal studies (e.g. Nevo *et al.*, 1984; Ward *et al.*, 1992). It is a particularly powerful method for solving taxonomic problems, and it has been used in several studies on lizards to quantify the divergence among populations or species (e.g. Blanc & Cariou, 1987; Busack & Maxson, 1987; Hutchinson & Schwaner, 1991; MacCulloch *et al.*, 1995; Martins, 1995; Bobyn *et al.*, 1996). In this study, both conventional electrophoresis and isoelectric focusing techniques were used for the first time to investigate the patterns of genetic diversity and the degree of genetic differentiation among populations of *L. schreiberi*.

MATERIAL AND METHODS

Samples were collected in summer (1994 and 1995) from 11 populations throughout the distribution area of the species (Fig. 1). Two hundred and forty-six individuals were caught, and the tail-tip of each was taken together with the blood that emerges from the cut. Both tail and blood were snap-frozen in liquid nitrogen and stored at -20°C. Animals were immediately released at the capture site.

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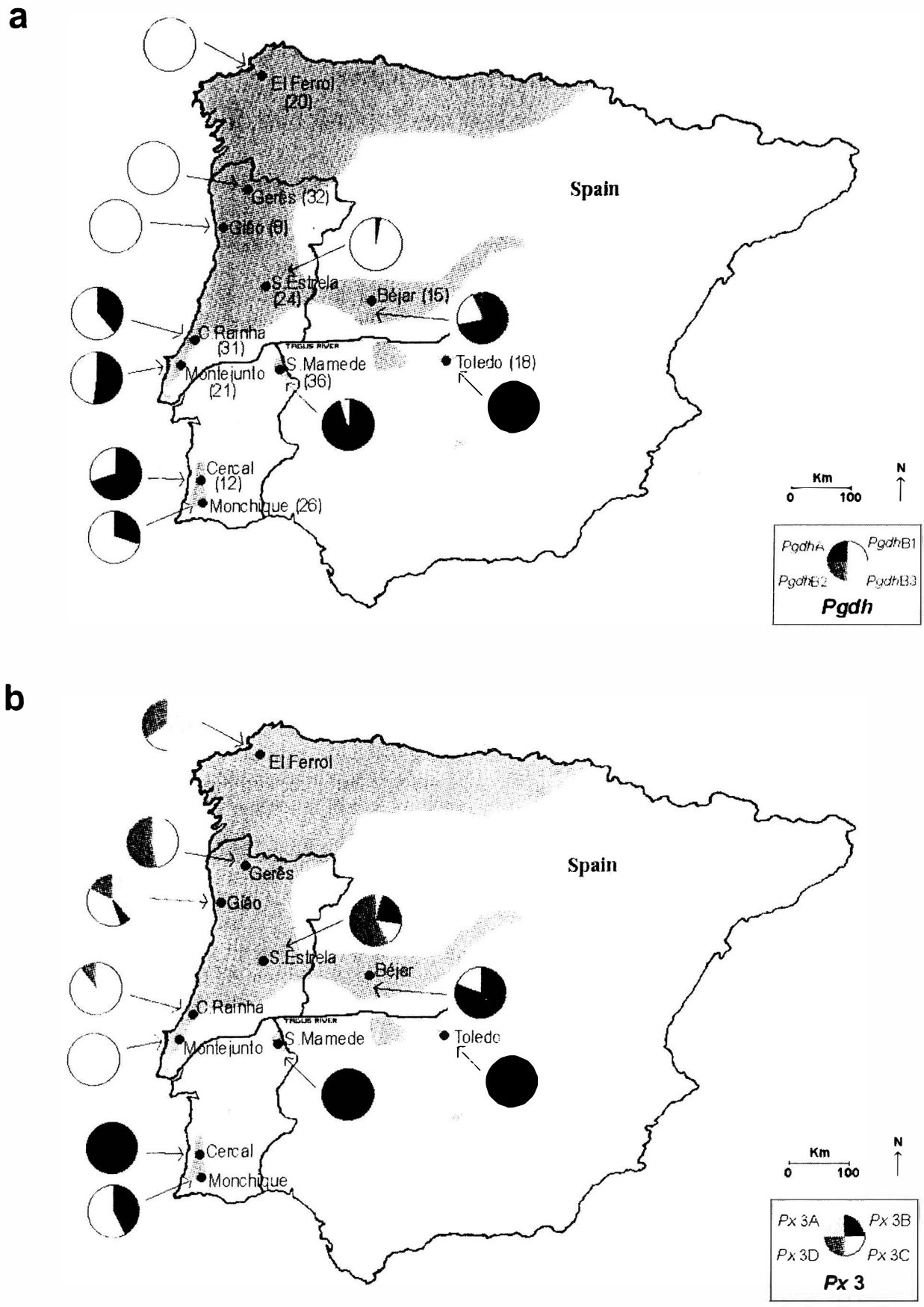

FIG 1. Distribution area of *Lacerta schreiberi*, location of sampling sites and the number of individuals sampled from each site (in parentheses). The pie charts represent the allele frequencies at the (a) *Pgdh* locus, (b) *Px*3 locus.

Tail samples were mechanically homogenized in an equal volume of Tris-EDTA-HCl buffer (pH 6.8) and centrifuged at 30 000 g for 30 min at 4°C. Supernatant fractions were subjected to horizontal electrophoresis using 13% starch gels, following the methods of Pasteur *et al.* (1987). Blood samples were diluted in equal volumes of distilled water and used directly in cellulose acetate gels (Gelman). Both tail and blood samples were used in isoelectric focusing. Twenty-one enzyme systems were consistently scored using electrophoresis and/or isoelectric focusing for each individual. Protein names, EC numbers and the technique with which the protein was screened are presented in Table 1. Enzymes are referred to in the text by their abbreviations in block letters, while enzyme loci are referred to by the italicised abbreviations in lower case.

Alleles of *Pep*B, *Pgdh*, *Px*2 and *Px*3 were resolved by isoelectric focusing in pH gradients established by the following mixtures of (1) 5-6 ampholyte, (2) 1:3 of 7-9 and 8-9.5 ampholytes, (3) 1:1 of 4.5-5.4 and 5-6 ampholytes, and (4) 1:1 of 3.5-5 and 4.5-5.4 ampholytes, respectively. All ampholytes were used at a final concentration of 6% (v/v). Anodal and cathodal electrode solutions were, respectively, (1) aspartic acid/glutamic acid 0.05M and sodium hydroxide 1M for PEPB and PGDH, (2) aspartic acid 0.04M and sodium hydroxide 0.6M for *Px2,* and (3) aspartic acid 0.04M and sodium hydroxide 0.2M for *Px*3. Gels were prefocused for 1 hr at constant power setting limits at 1500 V, 25 mA, and 1W (30 min), 2W (15 min) and 3 W

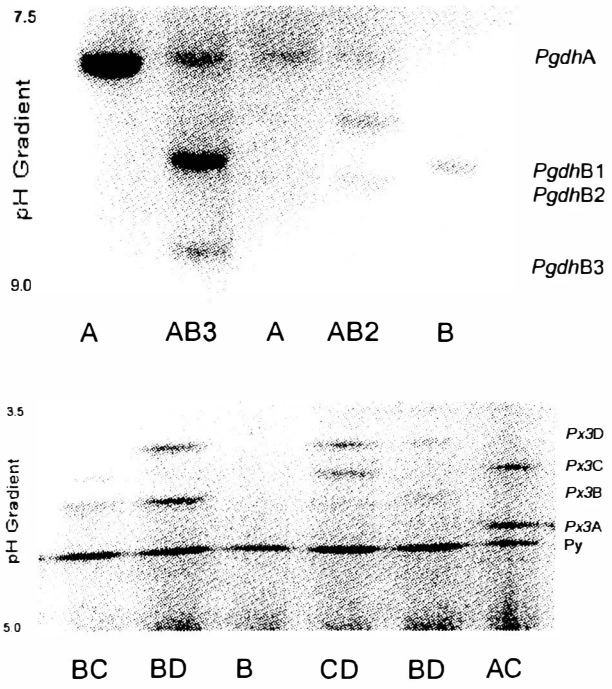

FIG 2. Electrophoretic patterns of *Pgdh* (top) and *Px*3 (bottom) revealed by isoelectric focusing. In the bottom figure appears another locus which is represented by *Py*.

(15 min). After prefocusing, 8 mL of sample were applied to the gels using a silicone strip (Serva). Focusing was then performed for 3 hr at constant power settings limits at 1500 V, 25 mA, and 4 W (1 h), 5 W (1 hr) and

TABLE 1. Enzyme systems, E.C. numbers and scoring techniques for each system. SGE - Starch gel electrophoresis: (1) Tris-citrate, pH 8.0; (2) Tris-citrate, pH 6.7; (3) LiOH-borate, pH 8.3; (4) Tris-NaH_2PO_4, pH 7.4. CAE - Cellulose acetate electrophoresis: Tris-borate, pH 7.6. AGE - Agarose gel electrophoresis: Tris-glycine, pH 8.6. IEF - Isoelectric focusing.

Enzyme system and locus	EC	Technique	System
Adenylate Kinase (*Ak*)	2.7.4.3	SGE	2
Albumine (*Alb*)	–	CAE	–
α-Amylase (*Amy*)	3.2.1.1	AGE	–
Aspartate Aminotransferase (*Aat*1; *Aat*2)	2.6.1.1	SGE	1
Creatine Kinase (*Ck*1; *Ck*2)	2.7.3.2	SGE	2
Esterase (*Est*, non specific)	3.1.1.-	SGE	3
Glucose-6-Phosphate Isomerase (*Gpi*)	5.3.1.9	SGE	1
Glycerol-3-Phosphate Dehydrogenase (*G3pdh*)	1.1.1.8	SGE	1
Lactate Dehydrogenase (*Ldh*1; *Ldh*2)	1.1.1.27	SGE	1
Malate Dehydrogenase (*Mdh*)	1.1.1.37	SGE	1
Malate Dehydrogenase-$NADP^+$ (*Me*)	1.1.1.40	SGE	2
Mannose-6-Phosphate Isomerase (*Mpi*)	5.3.1.8	SGE	1
Non specific protein (*Px*1)	–	SGE	2/3
Non specific protein (*Px*2)	–	IEF	–
Non specific protein (*Px*3)	–	IEF	–
Peptidase B (*Pep*B)	3.4.13.-	IEF	–
Peptidase D (*Pep*D)	3.4.13.9	SGE	4
Phosphoglucomutase (*Pgm*)	5.4.2.2	SGE	2
Phosphogluconate Dehydrogenase (*Pgdh*)	1.1.1.44	SGE/IEF	2/-
Nucleoside Phosphorylase (*Np*)	2.4.2.1	IEF	–
Superoxide Dismutase (*Sod*)	1.15.1.1	SGE	1

TABLE 2. Standard genetic variability measures for the 24 loci screened in this study: mean number of individuals per sample (*n*), mean number of alleles per locus (*a*), percentage of polymorphic loci ($P_{0.95}$), average observed heterozygosity (H_O) and average expected heterozygosity (H_E).

Population	*n*	*a*	$P_{0.95}$	H_O	H_E
Main distribution area					
El Ferrol	19.2	1.2	17.4	0.09	0.08
Gerês	25.8	1.3	21.7	0.07	0.08
Gião	13.0	1.2	8.7	0.04	0.03
Estrela	21.8	1.4	17.4	0.09	0.09
C.Rainha	27.2	1.3	30.4	0.09	0.09
Montejunto	20.5	1.3	26.1	0.07	0.08
Béjar	13.7	1.5	34.8	0.14	0.12
Isolated populations					
Toledo	15.3	1	0	0.00	0.00
S.Mamede	30.8	1.3	17.4	0.06	0.06
Monchique	25.6	1.2	17.4	0.05	0.05
Cercal	12.4	1.1	13.0	0.05	0.04
Average	20	1.3	18.6	0.07	0.07

6 W (1 hr). Allelic variation was visualized using histochemical techniques (Harris & Hopkinson, 1976).

Genetic variability of each population across 24 presumptive loci (*Np* was excluded from the analysis because it was considered a dominant/recessive locus) was characterized by the mean number of alleles per locus (*a*), the percentage of polymorphic loci (*P*) and the observed (H_O) and expected (H_E) average heterozygosity. For polymorphic loci, a chi-square test was used to check whether genotypic frequencies were in Hardy-Weinberg equilibrium. The magnitude of genetic differentiation was investigated with Weir & Cockerham (1984) estimators of *F*-statistics.

Cavalli-Sforza chord distances (Cavalli-Sforza & Edwards, 1967) were used to evaluate patterns of genetic differentiation and are presented in the form of a Neighbour Joining (NJ) tree (Saitou & Nei, 1987). Support for nodes was generated with bootstrap replicates (1000 trees). All calculations were performed using FSTAT version 2.9.1 (Goudet, 2000) and PHYLIP version 3.5 (Felsenstein, 1993) program packages.

RESULTS AND DISCUSSION

Thirteen out of the 24 presumptive structural loci scored were found to be polymorphic (frequency of most common allele was less than 99%) in at least one population, providing a total of 30 alleles. Although no progeny testing was performed to confirm the mode of inheritance of protein variants, zymograms conformed with simple patterns of codominant inheritance. The electrophoretic bands corresponding to the alleles identified at each locus were numbered according to their order of discovery.

Allelic frequencies of polymorphic loci are given in the Appendix 1. The two most informative loci corresponded to phosphogluconate dehydrogenase (*Pgdh*) and to a plasmatic protein (*Px*3), which received a detailed analysis (Fig. 1). While starch gel electrophoresis revealed the presence of only two alleles at the *Pgdh* locus (*Pgdh*A and *Pgdh*B), isoelectric focusing systems allowed the identification of three subtypes of *Pgdh*B (*Pgdh*B1, *Pgdh*B2 and *Pgdh*B3) (Fig. 2a). *Pgdh*B1 was fixed or almost fixed in the northern populations of El Ferrol, Gerês, Gião and S.Estrela, and showed high frequencies in central and southern Atlantic populations (Caldas da Rainha – 0.61, Montejunto – 0.48, Cercal – 0.30, and Monchique – 0.71). In contrast, this allele was absent in isolated inland populations (S. Mamede and Toledo) and occurred at low frequency (0.23) in a population from the Spanish Central System (Béjar) along the eastern edge of the species' main distribution area.

TABLE 3. *F*-statistics analysis for the polymorphic loci analysed in this study, using the Weir & Cockerham (1984) estimation of F_{IT} (F), F_{ST} (θ) and F_{IS} (f) and statistical significance of the values (NS, not significant; * $P<0.05$;** $P<0.01$; *** $P<0.001$).

Locus	*f*	*F*	θ
*Aat*1	0.003 NS	-0.000 NS	-0.003 NS
Amy	0.235*	0.449***	0.279***
*Ck*1	0.082 NS	0.525***	0.482***
Gpi	-0.017 NS	-0.001 NS	0.016*
*Ldh*1	-0.036 NS	-0.003*	0.032***
Me	0.054 NS	0.500***	0.472***
Mpi	-0.008 NS	-0.006 NS	0.001 NS
*Pep*B	-0.018 NS	0.106*	0.122***
*Pep*D	-0.076 NS	0.352***	0.397***
Pgdh	-0.106 NS	0.515***	0.562***
Pgm	-0.084 NS	-0.001*	0.076***
*Px*2	-0.158 NS	0.326***	0.418***
*Px*3	0.052 NS	0.527***	0.501***
All loci	0.025 NS	0.462***	0.448***

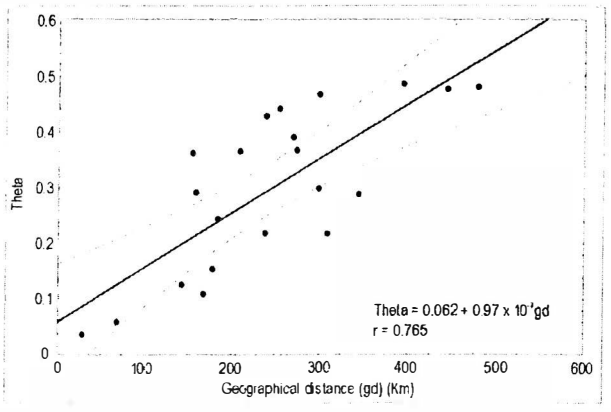

FIG 3. Regression of theta (θ) against geographical distance between pairs of populations of the main distribution area. The interrupted lines represent the bounds of the 95% confidence limits of the regression line.

The other major allele at this locus, *Pgdh*A, occurred at high frequencies in all southern populations including the isolate of S. Mamede, and reached fixation in Toledo. *Pgdh*B2 and *Pgdh*B3 were low-frequency private alleles from the populations of Béjar and S. Mamede, respectively. A second group of allelic frequencies corresponds to *Px*3, a non-specific plasmatic protein of acidic isoelectric point, showing four distinct alleles (Figs. 1 and 2b) with a well-established geographic distribution. Alleles *Px*3A and *Px*3D were present only in northern populations, where *Pgdh*A was fixed or highly predominant. *Px*3D was also present in Caldas da Rainha, although at a low frequency. *Px*3B was predominant in southern isolated populations, being the only allele present in Cercal, S. Mamede and Toledo. This allele was also present in the main distribution area, namely in Béjar (0.81) and S. Estrela (0.25). *Px*3C was almost present throughout the species' distribution, being absent only in the Cercal, S. Mamede and Toledo isolated populations.

Ten rare alleles ($P<0.10$) were found in six sampling regions, all of them included in the main distribution area of the species (central and northern Iberia). Of those, nine were private alleles (present in a single population) and only one (*Mpi*A) was shared at low frequencies by three different localities (Gerês, Béjar and S. Mamede). The population of Béjar harboured most of this diversity (five rare alleles), while two other populations (S. Estrela and S. Mamede) exhibited two rare alleles and three others (Gerês, Caldas da Rainha and Montejunto) a single one. These results are consistent with the biogeographical hypothesis described by Marco & Pollo (1993), suggesting an older age for *L. schreiberi* populations in the north-western corner of the Iberian Peninsula, and a recent post-glacial expansion to the south during periods of higher precipitation, followed by a population contraction and the persistence of isolated populations in meridional mountainous areas. Thus, the combination of population age with genetic drift phenomena during expansions may explain the present-day patterns of genetic diversity in this species.

Values of average H_E (7%) are consistent with average values reported generally for reptiles and other species of non-insular lizards (Nevo *et al.*, 1984; Ward *et al.*, 1992) (Table 2). When these measures are compared between *L. schreiberi* populations, it is remarkable that the two extreme values correspond to two geographically close locations: Béjar and Toledo. The first population showed the highest values for all the parameters ($P_{0.95}$=34.8%, a=1.5 and H_E=0.12), a fact that is in agreement with the long persistence of the species, in the Spanish Central System. On the other hand, the second population is characterized by a total lack of genetic variability, indicating that this isolate may have resulted from only a few founder individuals and/or that small population size has promoted genetic drift.

L. schreiberi exhibited a high degree of genetic substructure (F_{ST}=0.448) with most of the genetic variation existing among populations (Table 3). It is especially noteworthy that isoelectric focusing was responsible for revealing the two loci that most contribute to high population differentiation (F_{ST} of 0.562 and 0.501 for *Pgdh* and *Px*3, respectively). This is not surprising, because the high resolving power of this technique is well known (Righetti, 1990) and the effective separation of electromorphs into their constituent alleles necessarily generate higher F_{ST} values. Alternatively, selection at these loci or at closely linked loci may have increased differentiation, but only future work will clarify this issue. When populations from the main distribution area (El Ferrol, Gerês, Gião, S. Estrela, Caldas da Rainha, Montejunto and Béjar) and the southern isolates (Toledo, S. Mamede, Monchique and Cercal) are analysed separately, F_{ST} values change significantly, being much lower in the former set of populations (F_{ST} = 0.315) than in the second (F_{ST} = 0.582) (results not shown). This clearly suggests that populations in the southern isolates are more differentiated due to severe restrictions to gene flow coupled with small population numbers (Brito *et al.*, 1998). A more detailed analysis of F_{ST} values among populations is presented in Fig. 3 and Appendix 2. Consistent low levels of differentiation are found when populations included in the north-western main distribution area are compared (El Ferrol, Gerês, Gião and Estrela), probably indicating a more homogeneous population structure and fewer constraints to gene flow. This situation is also detected in the pair of marginal populations – C.Rainha/Montejunto – that are separated from the main distribution area by less than 150 km and are poorly differentiated. The relation between pairwise F_{ST} values and geographical distances for the populations on the main distribution area is depicted in Fig. 3 and fits a model for genetic structure of isolation-by-distance.

The Cavalli-Sforza distance-based NJ tree presents two main clusters corresponding to northern and southern samples (Fig. 4 and Appendix 2). The first group includes all northern populations from the main distribution area of *L. schreiberi* (93% bootstrap) that are known as large and continuous populations and have

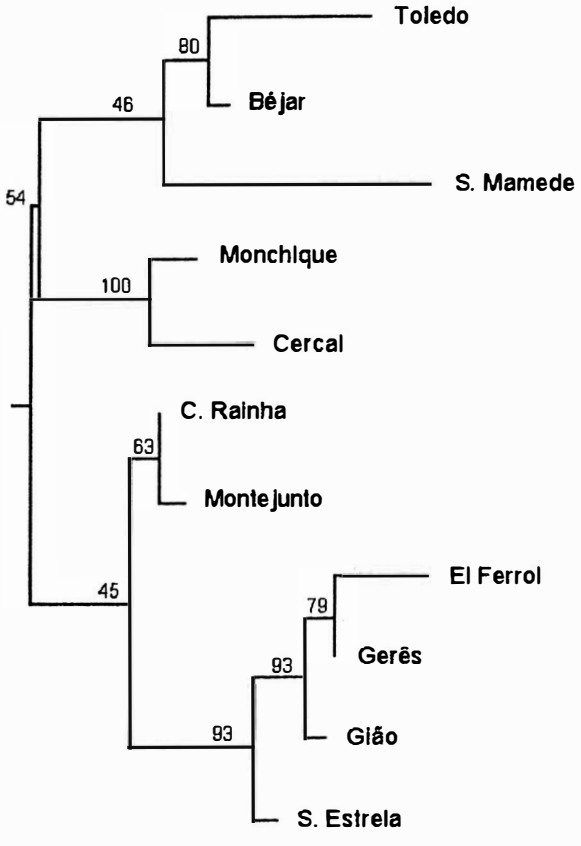

FIG 4. A neighbour-joining tree based on Cavalli-Sforza & Edwards (1967) chord genetic distance for 11 populations of *L. schreiberi*, with percent bootstrap replication scores indicated. The root was determined by estimating the midpoint between the two most divergent populations.

probably been connected by favourable habitat since at least the last glaciation. This set of populations cluster with the western Caldas da Rainha and Montejunto samples that correspond to two small isolated coastal populations. These populations have probably been isolated through human activities that fragmented habitats. These results are thus compatible with recent gene flow between Caldas da Rainha and Montejunto and the group of northern populations. The second group includes a central set of populations (Béjar, Toledo and S. Mamede), as well as the south-western isolates of Monchique and Cercal, and probably represents a history of fragmentation by a large ancestral southern population. This is clearly the case for Monchique and Cercal that are geographically distant from all other populations and still show similar allelic distribution profiles, thus reflecting a recent separation. In contrast, S. Mamede and Toledo probably result from two different expansion events originating in the Spanish Central System, resulting in very different genetic characteristics. In fact, while S. Mamede still exhibits moderate levels of H_E (0.06) and two rare alleles (*Pgdh*B3 and *Mpi*A), suggesting a relatively long history for this population, Toledo completely lacks genetic variability, indicating the occurrence of strong genetic drift.

Recent advances in molecular techniques and their application to a variety of organisms in the Iberian Peninsula have shown a complex combination of population histories, including fragmentations, contractions to glacial refugia, post-glacial expansions, and the formation of hybrid zones (Cooper *et al.*, 1995; Hewitt, 1996, 1999; Comes & Abbott, 1998; Alexandrino *et al.*, 2000; Branco *et al.*, 2000). In the future, a more detailed study comprising a considerable extension of the numbers of both genetic markers and sampled populations, as well as the use of hypervariable microsatellite and mtDNA markers, may certainly contribute to a better understanding of the recent evolution of *L. schreiberi* in the Iberian Peninsula.

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APPENDIX 1. Allelic frequencies of polymorphic loci in 11 populations of *L. schreiberi*.

Locus	Allele	El Ferrol	Gerês	Gião	Estrela	Toledo	Béjar	S. Mamede	Caldas Rainha	Monte-junto	Mon-chique	Cercal
*Aat*1	A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00
Amy	F	0.39	0.77	0.81	0.75	1.00	1.00	1.00	0.89	1.00	1.00	1.00
	S	0.61	0.23	0.19	0.25	0.00	0.00	0.00	0.11	0.00	0.00	0.00
*Ck*1	A	0.00	0.16	0.00	0.47	0.00	0.25	0.76	0.72	0.85	0.87	0.92
	B	1.00	0.84	1.00	0.53	1.00	0.75	0.24	0.28	0.15	0.13	0.08
Gpi	A	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
	B	1.00	1.00	1.00	0.96	1.00	0.97	1.00	1.00	1.00	1.00	1.00
	C	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*Ldh*1	A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
	B	1.00	1.00	1.00	1.00	1.00	0.93	1.00	0.95	1.00	1.00	1.00
	C	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
Me	A	0.22	0.06	0.00	0.20	0.00	0.73	0.85	0.71	0.53	0.98	0.83
	B	0.78	0.94	1.00	0.75	1.00	0.27	0.15	0.29	0.47	0.02	0.17
	C	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mpi	A	0.00	0.04	0.00	0.00	0.00	0.03	0.02	0.00	0.00	0.00	0.00
	B	1.00	0.96	1.00	1.00	1.00	0.97	0.98	1.00	1.00	1.00	1.00
Pep B	1	1.00	1.00	1.00	1.00	1.00	0.77	0.94	0.89	0.77	1.00	1.00
	2	0.00	0.00	0.00	0.00	0.00	0.23	0.06	0.11	0.23	0.00	0.00
Pep D	F	0.00	0.00	0.00	0.00	0.00	0.41	0.52	0.10	0.00	0.00	0.00
	S	1.00	1.00	1.00	1.00	1.00	0.59	0.48	0.90	1.00	1.00	1.00
Pgdh	A	0.00	0.00	0.00	0.04	1.00	0.70	0.97	0.39	0.52	0.29	0.70
	B1	1.00	1.00	1.00	0.96	0.00	0.23	0.02	0.61	0.48	0.71	0.30
	B2	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
	B3	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Pgm	A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00
	B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	1.00	1.00
*Px*2	A	0.41	0.70	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00
	B	0.59	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
*Px*3	A	0.50	0.09	0.37	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	B	0.00	0.00	0.06	0.25	1.00	0.81	1.00	0.00	0.00	0.40	1.00
	C	0.14	0.38	0.38	0.13	0.00	0.19	0.00	0.92	1.00	0.60	0.00
	D	0.36	0.53	0.19	0.58	0.00	0.00	0.00	0.08	0.00	0.00	0.00

APPENDIX 2. Below diagonal: Cavalli-Sforza & Edwards (1967) chord distance between populations; above diagonal: F_{ST} values per pair of population, using the Weir & Cockerham (1984) estimator θ.

	El Ferrol	Gerês	Gião	Estrela	Toledo	Béjar	S. Mamede	Caldas Rainha	Monte junto	Monc-hique	Cercal
El Ferrol	–	0.154	0.218	0.288	0.652	0.482	0.723	0.474	0.478	0.634	0.589
Gerês	0.071	–	0.060	0.110	0.575	0.440	0.675	0.366	0.466	0.556	0.550
Gião	0.124	0.080	–	0.125	0.717	0.390	0.690	0.363	0.426	0.621	0.603
Estrela	0.198	0.095	0.125	–	0.564	0.361	0.580	0.290	0.243	0.418	0.423
Toledo	0.543	0.460	0.352	0.353	–	0.400	0.647	0.625	0.530	0.756	0.812
Béjar	0.535	0.433	0.419	0.296	0.255	–	0.176	0.297	0.217	0.361	0.238
S. Mamede	0.703	0.593	0.608	0.374	0.282	0.094	–	0.465	0.275	0.472	0.231
C. Rainha	0.367	0.228	0.301	0.171	0.477	0.205	0.289	–	0.037	0.119	0.356
Montejunto	0.477	0.306	0.360	0.249	0.453	0.238	0.322	0.055	–	0.187	0.092
Monchique	0.504	0.363	0.403	0.218	0.442	0.189	0.213	0.107	0.119	–	0.277
Cercal	0.592	0.476	0.496	0.249	0.279	0.166	0.091	0.244	0.241	0.094	–