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ALLOZYME VARIATION AND POPULATION SUBSTRUCTURING IN THE CAUCASIAN GROUND LIZARDS *Lacerta derjugini* AND *Lacerta praticola*

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Genetic diversity at 35 allozyme loci was surveyed in *Lacerta derjugini* (3 populations) and *L. praticola* (2 populations). Indices of variability were consistent with those found in other Caucasian *Lacerta*. There was little genetic substructuring between two populations of *L. praticola* despite considerable geographic separation. Conversely, populations of *L. derjugini* in close proximity to one another exhibited considerable substructuring.

Key words: *Lacerta*, population genetics, protein electrophoresis, allozymes.

INTRODUCTION

The "ground lizards" *Lacerta derjugini* Bischoff and *L. praticola* Lantz et Cyrén, are interesting components of the Caucasus lizard fauna. The preferred habitat of these two species is ground vegetation, in either forested or open areas, whereas other small Caucasian *Lacerta* occupy rocky habitats. For this reason, among others, the two species have not been considered members of the speciose "rock lizard" group (Darevsky, 1967). Later investigations, however, found a close relationship among these two species and other Caucasian lacertas of the *saxicola* group (Arnold, 1989; Fu et al., in press; Grechko et al., 1993; Mayer and Lutz, 1989; Murphy et al., 1996a).

Recently, a preliminary phylogeny of Caucasian *Lacerta* based on allozymes found inconsistencies at several nodes (Murphy et al., 1996a). Some of these inconsistencies were due to the uncertainty of the phylogenetic relationships of the two ground lizard species *L. derjugini* and *L. praticola*. These two species must therefore be examined more closely in order to solidify their place in the evolutionary history of *Lacerta*. The first step toward this goal is a population-level allozyme assay of a broad geographic representation of a number of individuals of these species.

MATERIAL AND METHODS

Specimens of *Lacerta derjugini orlowae* were collected from Achaldaba, Bakuriani, and Zedazeni, Georgia. *Lacerta praticola pontica* were collected from Sochi and Tul'skaya, Krasnodar District, Russia. Specimens were euthanised by an overdose of sodium pentobarbital and dissected immediately following euthanasia. Liver, heart and skeletal muscle were removed and frozen in liquid nitrogen. Specimens are deposited in the herpetological collection of the Royal Ontario Museum (ROM); collection details and specimen numbers are available upon request.

Enzymes were separated by horizontal starch gel electrophoresis. All procedures, protocols and allelic nomenclature follow Murphy et al. (1996b). The analysis utilized 27 enzyme systems encoded by 35 presumptive loci. Wherever possible, gene products were resolved on two buffer systems to maximize expression of all variants. Enzyme names, EC numbers and specific buffer systems for the separation of locus

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products were the same as those used by Bobyn et al. (1996), Fu et al. (1995) and MacCulloch et al. (1995).

Allozyme data were analyzed using BIOSYS-1 release 1.7 (Swofford and Selander, 1989). The populations were treated separately for analyses of population variability. All loci were evaluated for three parameters of genetic polymorphism: heterozygosity (MHD), number of alleles per locus (MNA) and percentage of loci which were polymorphic (PLP), as well as conformity to Hardy-Weinberg expectations using Levene's (1949) correction for small sample sizes. The populations of each species were examined together for genetic structuring using Wright's (1978) *F*-statistics. Although only one specimen of *L. derjugini* was collected at Zedazeni, it was included in the analysis.

TABLE 1. Genotype Frequencies for the Polymorphic Loci in *Lacerta derjugini*

Locus	Population		
	Achaldaba	Bakuriani	Zedazeni
sAat-A	aa (10)	aa (8)	ab (1)
	ab (1)	ab (2)	
sAcoh-A	aa (10)	aa (6)	aa (1)
	ab (1)	ab (4)	
Cat-A	aa (7)	aa (7)	aa (1)
	ab (3)	ab (3)	
	ac (1)		
Ck-C	aa (8)	aa (10)	aa (1)
	ab (3)		
Gpi-A	aa (11)	aa (7)	aa (1)
		ab (3)	
sIdh-A	aa (11)	aa (9)	aa (1)
		ab (1)	
Pep-B	aa (11)	aa (8)	aa (1)
		ab (2)	
Pnp-A	aa (7)	aa (9)	aa (1)
	ab (3)	ab (1)	
	bb (1)		
N[1]	11	10	1
MHD[2]	0.033 ± 0.016	0.044 ± 0.017	0.028 ± 0.028
MNA[3]	1.17 ± 0.07	1.19 ± 0.07	1.03 ± 0.03
PLP[4]	8.33	19.44	2.78

[1] Number of individuals examined.
[2] Mean heterozygosity by direct count.
[3] Mean number of alleles per locus.
[4] Percentage of loci polymorphic (0.95 criterion).

RESULTS

Lacerta derjugini

All individuals from all three populations expressed homozygous allelic products at 27 of the 35 loci: mAat-A, mAcoh-A, Acp-B, Ada-A, Cbp-1, Ck-A, Est-D, βGa-1, Gcdh-A, Gda-A, βGlur-A, βGlus-A, Gpi-B, G6pdh-A, mIdh-A, Ldh-A, Ldh-B, mMdh-A, sMdh-A, mMdhp-A, sMdhp-A, Mpi-A, Pep-A, Pgm-A, mSod-A, sSod-A and Tpi-A. Genotype frequencies for the eight polymorphic loci are shown in Table 1. Only one locus (sAat-A) was polymorphic in all three populations. At four of the eight polymorphic loci, only one population exhibited polymorphism. In many cases polymorphism resulted from the single appearance of a rare allele. At four of the five loci which were polymorphic in more than one population, all alleles were present in all polymorphic populations. The sole exception was Cat-A, where Cat-A (c) made a single appearance in the Achaldaba population. The indices of genetic variability are shown at the bottom of Table 1. The analysis of *L. derjugini* included a single individual from Zedazeni. While it is unusual to use a sample size of one for a study of genetic variability, this individual was included because discrete populations often exhibit rare alleles, although in this instance none was found.

Allele frequencies at one locus failed to conform to Hardy-Weinberg probabilities. The Achaldaba population exhibited a slight heterozygote excess at Pnp-A.

The *F*-statistics for the three populations are as follows: $F_{IS} = -0.254$, $F_{IT} = 0.203$, $F_{ST} = 0.365$. The negative value of F_{IS} is indicative of an intrapopulational heterozygote excess, while the positive value of F_{IT} indicates an interpopulational heterozygote deficiency smaller than the intrapopulational excess. The relatively high positive value of F_{ST} suggests that the three populations do not form a panmictic group.

Lacerta praticola

All individuals in both populations of *L. praticola* were monoallelic for 20 of the 35 loci: mAat-A, mAcoh-A, Acp-B, Ada-A, Cbp-1, Ck-A, Ck-C, Est-D, βGa-1, Gcdh-A, Gda-A, βGlur-A, βGlus-A, Gpi-B, mMdh-A, mMdhp-A, Pgm-A, mSod-A, sSod-A and Tpi-A. Genotype frequencies for the 15 polymorphic loci are shown in Table 2. In most cases polymorphism was due to rare alleles. Of the 15 vari-

able loci, only four (sAat-A, Cat-A, Gpi-A, Pep-A) were polymorphic in both populations. At all four of these loci, the Sochi population exhibited a rare allele which was not detected in the sample from Tul'skaya. The Sochi population also exhibited polymorphism at nine loci which were monomorphic in the Tul'skaya population, whereas the Tul'skaya population exhibited polymorphism at only two loci which were invariant at Sochi. The difference in variability between the two populations may have resulted from the unequal sample sizes.

All allele frequencies conformed to Hardy-Weinberg equilibrium with the exception of Pep-B in the Sochi population, which exhibited a heterozygote deficiency. Indices of genetic variability are shown at the bottom of Table 2.

The *F*-statistics for the two populations are: $F_{IS} = -0.002$, $F_{IT} = 0.023$, $F_{ST} = 0.025$. These values are the lowest of any so far reported in Caucasian *Lacerta*. F_{IS} measures intrapopulational heterozygote deficiency or excess; it is close to zero. The positive value of F_{IT} indicates a small interpopulational heterozygote deficiency. The very low positive value of F_{ST} suggests that the two populations could form a panmictic group.

DISCUSSION

Values of MHD, MNA, and PLP of *L. derjugini* and *L. praticola* approximate those of other Caucasian *Lacerta* (*L. caucasica* and *L. daghestanica*, Fu et al., 1995; *L. raddei* and *L. nairensis*, Bobyn et al., 1996; *L. portschinskii, L. rudis*, and *L. valentini*, MacCulloch et al., 1995, in press). Heterozygosity in *L. derjugini* and *L. praticola* are similar to the values found in *Podarcis sicula* and *P. melisellensis* from large island and mainland populations (Gorman et al., 1975). Much higher levels of heterozygosity were found in *L. lepida* (Busack, 1987) and in the teiid lizard species *Cnemidophorus tigris* (Gorman et al., 1977).

A study of 10 genera found mean heterozygosity of 0.01 in fossorial lizards, 0.05 in "sit and wait" species and 0.09 in vagile species (Gorman et al., 1977). Heterozygosity in the two species examined in this study resembles that of "sit and wait" lizards, as did heterozygosity values in other bisexual Caucasian *Lacerta* (Bobyn et al., 1996; Fu et al., 1995; MacCulloch et al., 1995; in press). A more extensive summary reported a mean heterozygosity of 0.051 from 71 lizard taxa or populations, most of whom are vagile or "sit-and-wait" (Sattler and Ries, 1995). This number is also similar to that found in Caucasian *Lacerta*.

TABLE 2. Genotype Frequencies for the Polymorphic Loci in *Lacerta praticola*

Locus	Population	
	Sochi	Tul'skaya
sAat-A	aa (38)	aa (19)
	ab (3)	ab (2)
	ac (2)	
sAcoh-A	aa (42)	aa (21)
	ab (1)	
Cat-A	aa (32)	aa (19)
	ab (4)	ac (2)
	bb (1)	
	ac (6)	
Gpi-A	aa (38)	aa (19)
	ab (3)	ac (2)
	ac (2)	
G6pdh-A	aa (42)	aa (21)
	ab (1)	
s-Idh-A	aa (41)	aa (21)
	ab (2)	
m-Idh-A	aa (43)	aa (19)
		ab (2)
Ldh-A	aa (42)	aa (21)
	ab (1)	
Ldh-B	aa (41)	aa (21)
	ab (2)	
s-Mdh-A	aa (40)	aa (21)
	ab (3)	
s-Mdhp-A	aa (42)	aa (21)
	ab (1)	
Mpi-A	aa (41)	aa (21)
	ab (2)	
Pep-A	aa (41)	aa (16)
	ab (2)	ac (5)
Pep-B	aa (39)	aa (21)
	ab (3)	
	bb (1)	
Pnp-A	aa (43)	aa (19)
		ab (2)
N	43	21
MHD	0.025 ± 0.009	0.015 ± 0.008
MNA	1.46 ± 0.11	1.11 ± 0.05
PLP	11.43	2.86

Note. For designations see Table 1.

In our study the percentage of loci exhibiting polymorphism was calculated using the 0.95 criterion in BIOSYS-1. Since some studies did not use this criterion, PLP was recalculated without the criterion to facilitate comparison. This produced a different value in the Achaldaba sample of *L. derjugini* (13.89%) and in both samples of *L. praticola* (37.14 and 11.43% at Sochi and Tul'skaya, respectively); the other populations did not change. Other Caucasian *Lacerta* exhibited similar values. Mainland populations of *P. sicula* had PLP of 27 – 45% (Gorman et al., 1975), while *L. lepida* had PLP of 23.1 and 38.5% (Busack, 1987). PLP varied from 10 – 21% in three species of bisexual *Cnemidophorus* (Dessauer and Cole, 1984).

The range of *L. praticola* is much more extensive than that of *L. derjugini*. Range size has been shown to be positively correlated with the amount of genetic variability in lacertids (Gorman et al., 1975; MacCulloch et al., in press). The same correlation is apparent in this study.

Comparison of the values of F_{ST} of the two species shows that *L. derjugini* has a greater value than does *L. praticola* (0.365 vs. 0.025). F_{ST} is an index of genetic substructuring among populations; the higher the value of F_{ST} the less likelihood that the populations constitute a panmictic group. Comparison of the relative values of F_{ST} in the two species to the distances between sampling sites provides insight into population substructuring. Even though the two sampling locations of *L. praticola* are separated by more than 100 km and a mountain range, F_{ST} in *L. praticola* differs little from zero. This very low value, lower than in any other Caucasian *Lacerta* (Bobyn et al., 1996; Fu et al., 1995; MacCulloch et al., 1995, in press), suggests that gene flow among populations of this species remains high despite geographic obstacles. The samples of *L. derjugini* were approximately the same distance apart, but there were no obvious physical barriers separating them. Rather, because the three locations were all from within the same river valley system, gene flow should be facilitated among local populations. Despite this, *L. derjugini* exhibited a much higher F_{ST} than did *L. praticola*. Because two of the sampling locations for *L. derjugini*, Achaldaba and Bakuriani, are only some 20 km apart, F_{ST} was recalculated for these two samples alone. The resulting value of 0.288, although lower than that calculated from three populations, is still one order of magnitude greater than F_{ST} in *L. praticola* and is greater than F_{ST} in any other Caucasian *Lacerta* from within a contiguous distribution. This suggests either the presence of an unreported barrier(s) to gene flow in *L. derjugini* along the Kura river valley system, or some other mechanism which produces genetic substructuring in the species.

While *L. derjugini* and *L. praticola* share the "ground lizard" microhabitat preference, this niche may have been independently derived in each species from the typical rocky habitat preferred by most small species of Caucasian *Lacerta*. This likelihood is supported by the two species' evolutionary relationships. Although the two species are grouped phylogenetically with the Caucasian rock lizards, they were not resolved as sister taxa (Fu et al., in press; Murphy et al., 1996a).

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