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Disjunct habitats as islands: genetic variability in the Caucasian rock lizard *Lacerta portschinskii*

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Abstract

Genetic diversity at 37 allozyme loci was surveyed in *Lacerta portschinskii* from contiguous populations and from a disjunct population. Indices of genetic diversity (heterozygosity, number of alleles per locus, and percentage of loci polymorphic) were greater in contiguous populations than in the smaller disjunct population. In this regard, disjunct populations appear to be similar to island populations. Indices of genetic diversity in Caucasian *Lacerta* are less than those reported from vagile lizard taxa and more similar to those of sit-and-wait predators.

Introduction

One factor affecting the amount of intrapopulation genetic variation is the size of the population. Reduction in genetic variability has been attributed to restriction of population size by geographic barriers and to founder effect. In one study, indices of genetic variability (heterozygosity and percentage of loci exhibiting polymorphism) in Adriatic island and mainland lacertid lizards (*Podarcis*) were found to be greater in mainland populations than in island populations (Gorman et al., 1975). Island size was also found to be positively correlated with variability, with indices of genetic variability of populations on larger islands approaching the values exhibited by mainland populations.

The greatest taxonomic diversity of the lizard genus *Lacerta* occurs in the Caucasian region (Darevsky, 1967). The varied topography and vegetation of the region have influenced the distributions of many species of *Lacerta*, producing some patchy, disjunct distributions.

An earlier study examined genetic variability in three species of Caucasian *Lacerta* (MacCulloch et al., 1995a). Species collected from restricted, disjunct populations (*L. portschinskii, L. valentini*) exhibited lower values of genetic variability than did the species collected from a contiguous population (*L. rudis*). Although interspecific, the comparison was of some value because these three species are closely related, forming a well-supported clade in the phylogeny of *Lacerta* (Fu et al., in press; Murphy et al., 1996a).

Recently, *L. portschinskii* were collected in central Georgia, in the largest, contiguous portion of the species' range (Darevsky, Kupriyanova & Uzzell, 1985; MacCulloch et al., 1995a). This permitted a direct, intraspecific test of the hypothesis that geographically restricted disjunct populations are less variable genetically than are contiguous populations and thus resemble island populations, which are less variable than mainland populations.

Materials and methods

Specimens were collected from three locations: Zedazeni, Manglisi, and Kodjori, within the largest, contiguous range of *Lacerta portschinskii* in central Georgia during 1995 (Figure 1). Specimens were

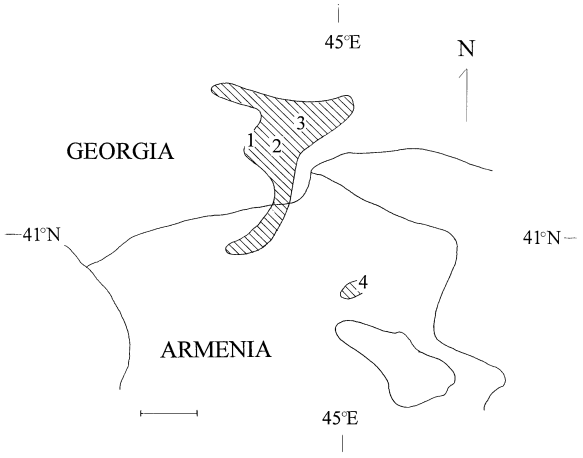

Figure 1. Distribution of *Lacerta portschinskii* (hatched area). Numbers represent collecting locations: (1) Manglisi; (2) Kodjori; (3) Zedazeni; (4) Gosh. The bar at the lower left represents 25 km.

euthanised by an overdose of sodium pentobarbitol 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; Appendix).

Enzymes were separated by horizontal starch gel electrophoresis. All procedures, protocols, and allelic nomenclature follow Murphy et al. (1996b). The analysis utilized 28 enzyme systems encoding 37 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 products were the same as those used by Bobyn et al. (1996), Fu et al. (1995), and MacCulloch et al. (1995a). Results obtained from the three Georgian populations were compared to the values obtained from a sample from Gosh, Armenia (Appendix), part of a disjunct population of *L. portschinskii* (MacCulloch et al., 1995a). Alleles from the Gosh sample and from the three Georgian samples were tested for identity in side-by-side comparisons.

Allozyme data were analysed using BIOSYS-1 release 1.7 (Swofford & 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), and conformity to Hardy-Weinberg expectations using Levene's (1949) correction for small sample sizes. The populations were examined together for genetic structuring using Wright's (1978) F statistics. The F statistics were calculated for all four populations together and for the three Georgian populations alone.

Results

All four populations were monomorphic for 26 of the 37 loci: mAat-A, Ada-A, Cbp-1, Ck-A, Est-D, βGa-1, βGa-2, Gcdh-A, Gda-A, βGlur-A, βGlus-A, Gpi-A, sIdh-A, Ldh-A, Ldh-B, mMdh-A, sMdh-A, sMdhp-A, Mpi-A, Pk-A, Pnp-A, mSod-A, sSod-A, Tpi-A. Genotype frequencies for the 11 polymorphic loci are shown in Table 1. Only one locus (Pep-B) was polymorphic in all four populations. At five of the 11 polymorphic loci, only one population exhibited polymorphism; in most cases this resulted from a single appearance by a rare allele.

At six of the polymorphic loci, at least one of the populations from Georgia exhibited variation, whereas the sample from Gosh was monoallelic. At two loci (mIdh-A and Pgm-A) the Gosh sample exhibited variation whereas all three Georgian samples were monoallelic. Allele frequencies at six loci failed to conform to Hardy-Weinberg probabilities. An excess of heterozygotes was exhibited at Acp-B in Zedazeni and sAcoh-A at Manglisi while mAcoh-A in Manglisi, sAcoh-A, mAcoh-A, and Gpi-B in Kodjori exhibited heterozygote deficiencies.

The indices of genetic variability are shown at the bottom of Table 1. The values of all indices are greater in the three Georgian samples than in the Gosh sample.

The F statistics for the four populations are as follows: $F_{IS} = 0.075$, $F_{IT} = 0.365$, $F_{ST} = 0.313$. Positive values of F_{IS} and F_{IT} are indicative of heterozygote deficiencies. The low value of F_{IS} indicates a slight intrapopulational deficiency. The higher value of F_{IT} shows an interpopulational heterozygote deficiency greater than the intrapopulational deficiency. The relatively high positive value of F_{ST} suggests that the four populations do not form a panmictic group. The F-statistics for the three Georgian populations are $F_{IS} = 0.079$, $F_{IT} = 0.193$, $F_{ST} = 0.123$. The first demonstrates a slight intrapopulational heterozygote deficiency virtually identical to that of the four populations. The values of both F_{IT} and F_{ST} are much lower than those calculated for the four populations. The lower F_{IT} shows that removal of the less variable disjunct Gosh

Table 1. Genotype frequencies for polymorphic loci in the four populations of *Lacerta portschinskii*

	Gosh	Zedazeni	Manglisi	Kodjori
sAat-A	aa(39)	aa(29)	aa(8)	aa(5)
				ab(1)
sAcoh-A	aa(39)	aa(7)	ab(6)	aa(1)
		ab(13)	bb(2)	bb(3)
		ac(1)		ac(1)
		bb(8)		
mAcoh-A	aa(39)	aa(17)	aa(4)	aa(3)
		ab(9)	bb(4)	ab(1)
		bb(2)		bb(2)
Acp-B	aa(39)	aa(19)	aa(7)	aa(3)
		ab(10)	ab(1)	ab(2)
Cat-A	aa(38)	ab(3)	bb(4)	bb(6)
	ab(1)	bb(25)	bc(4)	
		bc(1)		
Ck-C	aa(39)	aa(26)	aa(8)	aa(6)
		ab(3)		
Gpi-B	aa(39)	aa(29)	aa(8)	aa(5)
				bb(1)
mIdh-A	aa(38)	aa(29)	aa(8)	aa(6)
	ab(1)			
Pep-A	aa(33)	aa(29)	aa(2)	aa(6)
	ab(6)		ab(4)	
			ac(2)	
Pep-B	aa(31)	aa(13)	aa(3)	ab(2)
	ab(7)	ab(7)	ac(4)	bb(2)
	bb(1)	bb(1)	cc(1)	
		ac(6)		
Pgm-A	aa(37)	aa(29)	aa(8)	aa(6)
	ab(2)			
MHD[a]	.011	.051	.071	.039
MNA[b]	1.11	1.24	1.19	1.19
PLP[c]	5.56	16.22	16.22	16.22

[a]MHD = mean heterozygosity by direct count.
[b]MNA = mean number of alleles per locus.
[c]PLP = percentage of loci polymorphic (0.95 criterion).

population reduces the interpopulational heterozygote deficiency. The lower F_{ST} indicates that the three Georgian populations are much more likely to constitute a panmictic group than are the four populations. However, this F_{ST} is still significant ($p < 0.05$), even when corrected for sample size (Workman & Niswander, 1970), demonstrating that the three populations are not panmictic.

Discussion

Disjunct habitats as islands

Comparison of the three indices of genetic variability shown in Table 1 supports the hypothesis that isolated, disjunct populations exhibit less genetic variation than do larger, contiguous populations. The sample from the disjunct Gosh population has lower values of all three parameters of variability. This demonstrates that terrestrial habitat that is unoccupied, for whatever reason, by a terrestrial species is as effective a barrier to gene flow as is water to insular species.

Lacerta portschinskii prefers river valley habitat; the disjunct population is separated by mountains from the southern limit of the species' contiguous range. Gosh is approximately 50 km from the southern limit of the contiguous range and 100 km from the nearest Georgian sampling site. It is not known how long the Gosh population has been isolated; the Caucasus region has undergone many postglacial microhabitat changes, of both natural and human origin, and faunal distributions have changed accordingly. The lower genetic variability could have resulted from a loss of variability in a relict population or from the 'bottleneck' effect of a founder event.

Comparison with other taxa

Values of MHD, MNA, and PLP of *L. portschinskii* from the contiguous portion of its range approximate those of other Caucasian *Lacerta* sampled from contiguous ranges *(L. caucasica* and *L. daghestanica*, Fu et al., 1995; *L. raddei* and *L. nairensis*, Bobyn et al., 1996; *L. rudis*, MacCulloch et al., 1995a). The disjunct population of *L. portschinskii* has genetic variability parameters equivalent to those found in *L. valentini*, a high-elevation species with patchy distribution (MacCulloch et al., 1995a).

Heterozygosity in *L. portschinskii* from the contiguous part of its range is also comparable to that 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, Kim & Taylor, 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, Kim & Taylor, 1977). Heterozygosity in *Lacerta portschin-*

skii more closely resembles that of the 'sit-and-wait' group. Similar low heterozygosity values were reported for other bisexual Caucasian *Lacerta* (Bobyn et al., 1996; Fu et al., 1995; MacCulloch et al., 1995a). A more extensive summary reported a mean heterozygosity of 0.051 from 71 lizard taxa or populations, most of which are relatively vagile (Sattler & Ries, 1995). This number is closer to that found in Caucasian *Lacerta.*

In our study, the percentage of loci exhibiting polymorphism was calculated using the 0.95 criterion in BIOSYS-1. Because some studies did not use this criterion, PLP was recalculated without the criterion to facilitate comparison. This produced a different value in the Gosh sample only (PLP = 13.51%); the other populations remained at 16.22%. 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 & Cole, 1984).

Lacerta portschinskii is one of the parental species of the complex of parthenogenetic *Lacerta.* Among the parthenogenetic species so far examined, clonal diversity is low (Fu et al., in press; MacCulloch et al., 1995b; 1997; Murphy et al., in press). Study of the genetic variability of the parental species is an important part of understanding the origins and diversity of parthenogenetic *Lacerta*.

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Appendix

Specimens examined: Georgia, Zedazeni, 41° 50′ N, 044° 43′ E, ROM 26677–26705 (29 specimens); Georgia, Manglisi, 41° 43′ N, 044° 25′ E, ROM 26669–26676 (8 specimens); Georgia, Kodjori, 41° 38′32″ N, 044° 41′02″ E, ROM 26663–26668 (6 specimens); Armenia, Gosh, 40° 44′51″ N, 045° 01′26″ E, ROM 23926–23940, 24854–24878 (39 specimens).

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