Clonal variation in the parthenogenetic rock lizard *Lacerta armeniaca*

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Abstract: Genetic diversity at 35 allozyme loci was surveyed in seven populations of *Lacerta armeniaca*. Fixed heterozygotes were present at 16 loci, with homozygotes at 17 loci. Variation occurred at two loci, one in each of two populations, indicating one widespread clone, one restricted clone, and one apparently restricted clone. The low level of variation in this species suggests a recent restricted origin, involving few parental individuals.

Key words: *Lacerta*, allozymes, parthenogenesis.

Résumé : La diversité génétique chez sept populations du *Lacerta armeniaca* a été déterminée au niveau de 35 loci alloenzymatiques. Des hétérozygotes fixés ont été observés pour 16 des loci et des homozygotes fixés pour 17 des loci. La variation a été observée au niveau de deux loci, chacun dans une population différente. Ces résultats indiquent la présence d'un clone très répandu, d'un clone très restreint et d'un dernier clone apparemment très restreint. Le faible degré de variation chez cette espèce suggère une origine récente et limitée à quelques individus parentaux.

Mots clés : *Lacerta*, alloenzymes, parthénogenèse.

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Introduction

Parthenogenesis in vertebrates is well known but not well investigated. Typically, parthenogenetic vertebrates are all female, of hybrid origin, and reproduce clonally (Dawley 1989).

Recent investigations have shown that all diploid parthenogenetic species investigated thus far exhibit some degree of diversity in their genetic makeup, as well as an expected high level of fixed heterozygosity (Bezy and Sites 1987; Dessauer and Cole 1989; Donnellan and Moritz 1995; Parker and Selander 1984). Possible sources of this variation are either mutation, multiple origins, or genetic recombination (Cole et al. 1988; Moritz et al. 1989; Parker 1979). Further study of the origins of hybrid unisexual lizards suggests that the amount of allozyme variation can be correlated with both the number of individuals of the parental species involved in the origins and with the size of the area of origin (Moritz 1991; Moritz et al. 1992). The amount of variation may also be related to the ecology, distribution, and age of parthenogenetic taxa (Dessauer and Cole 1989; Parker et al. 1989).

Among the reptiles, parthenogenesis has been documented in seven families (Vrijenhoek et al. 1989); the first family in which this phenomenon was discovered was the Lacertidae (Darevsky 1958). The Caucasus rock lizards, genus *Lacerta* L., are of systematic interest because of the abundance of parthenogenetic forms occurring among them. To date, seven unisexual species of *Lacerta* have been discovered (Darevsky et al. 1985; Schmidtler et al. 1994). The parental species involved in the production of *Lacerta armeniaca* are *L. mixta* ♀ and *L. valentini* ♂ (Darevsky 1967; Moritz et al. 1992; Uzzell and Darevsky 1975; R.D. MacCulloch, unpublished data). The parental species are members of different clades in the subgenus *Archaeolacerta* and are separated by a Nei's (1978) genetic distance of 0.774–0.778 (R.W. Murphy, unpublished data).

Reproduction in *L. armeniaca* is more complex than simple parthenogenesis. *Lacerta armeniaca* has been found to produce occasional males (Darevsky et al. 1978) and to breed successfully with both males of *L. valentini* (Darevsky and Danielyan 1968) and rare triploid males of *L. unisexualis* (Darevsky et al. 1989).

An analysis of five allozyme loci in *L. armeniaca* by Uzzell and Darevsky (1975) found that all individuals were heterozygous at one creatine kinase locus (presumably *Ck-C*; Murphy and Crabtree 1985) and at the *Mpi-A* locus.

Little attention has been given to the potential for multiple clones in parthenogenetic *Lacerta*. Given the presence of more than one clone among other diploid parthenogenetic reptiles examined thus far, we investigated the amount of genetic variation among several populations of *L. armeniaca*.

Materials and methods

Specimens were collected in seven locations in central and northern Armenia (Appendix), in the central or southwestern

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Table 1. Summary of genetic variability coefficients for the seven populations of *L. armeniaca*.

	Papanino	Ankavan	Sevan	Stepanavan	Sevan Pass	Kutchak	Tumanyan
N	27	28	6	4	7	2	1
MHD	0.494 (0.085)	0.514 (0.086)	0.514 (0.086)	0.514 (0.086)	0.510 (0.085)	0.486 (0.086)	0.514 (0.086)
MNA	1.51 (0.09)	1.51 (0.09)	1.51 (0.09)	1.51 (0.09)	1.51 (0.09)	1.49 (0.09)	1.51 (0.09)
PLP	51.43	51.43	51.43	51.43	51.43	48.57	51.43

Note: Numbers in parentheses are standard errors. *N*, number of specimens; MHD, mean heterozygosity by direct count; MNA, mean number of alleles per locus; and PLP, percentage of loci that were polymorphic (0.95 criterion).

part of the species' range (Darevsky et al. 1985). Specimens were euthanised by an overdose of sodium pentobarbitol and dissected immediately. Liver, heart, and skeletal muscle were removed and frozen in liquid nitrogen. Some juvenile specimens were frozen whole and dissected later. Voucher specimens are deposited in the herpetological collection of the Royal Ontario Museum (ROM).

Genetic diversity was determined by protein electrophoresis. All electrophoretic procedures and protocols and enzyme, locus, and allele nomenclature follow Murphy et al. (1995). Our survey included 28 enzyme systems encoding 35 presumptive gene loci. Wherever possible, loci were resolved on two buffer systems to ensure that any variants were expressed. Specific buffer systems for the electrophoretic separation of locus products were the same as those used by Fu et al. (1995) and MacCulloch et al. (1995).

The allozyme data were analysed using BIOSYS-1 release 1.7 (Swofford and Selander 1989). All loci were evaluated for heterozygosity, number of alleles per locus and percent of loci that were polymorphic.

Results

Of the 35 loci examined, 16 (*sAat-A*, *sAcoh-A*, *mAcoh-A*, *Acp-B*, *Cat-A*, *Ck-C*, *Est-D*, *Gcdh-A*, *Ldh-B*, *mMdh-A*, *mMdhp-A*, *sMdhp-A*, *Mpi-A*, *Pep-A*, *Pep-B*, and *sSod-A*) were heterozygous in all individuals in all populations (Table 1). All individuals in all populations were homozygous at 17 loci (*mAat-A*, *Ada-A*, *Cbp-1*, *Ck-A*, *Gda-A*, *βGlus-A*, *βGlur-A*, *Gpi-A*, *Gtdh-A*, *G6pdh-A*, *mIdh-A*, *Ldh-A*, *sMdh-A*, *Pgm-A*, *Pk-A*, *mSod-A*, and *Tpi-A*).

At two loci, *Pnp-A* and *sIdh-A*, some individuals exhibited variation. At *Pnp-A*, all individuals in the Ankavan, Sevan, Stepanavan, Sevan Pass, Kutchak, and Tumanyan populations were heterozygous. However, in the Papanino population, 8 individuals were heterozygous (*ab*) and 19 were homozygous for the relatively slower (*b*) allele. Similarly, at *sIdh-A*, all individuals other than those from Kutchak were heterozygous (*ab*), while both individuals from Kutchak were homozygous for the slower (*b*) allele.

Discussion

All loci but two in *L. armeniaca* exhibited fixation of alleles in a homozygotic or heterozygotic state, as expected in a parthenogenetic species. The observed variation in genotypes at two loci could have arisen from recombination, mutation, or multiple origin (Moritz et al. 1989; Parker 1979). The multiple origin explanation is not supported by the evidence from *sIdh-A*, because the paternal species *L. valentini* ($N = 59$) possesses only the *sIdh-A* (*b*) allele, while the maternal species *L. mixta* ($N = 7$) possesses only the *sIdh-A* (*a*) allele (MacCulloch et al. 1995; R.W. Murphy unpublished data). Although the presence of the (*b*) allele in *L. mixta* is not impossible, recombination or mutation is likely the cause of the homozygous condition in the specimens from Kutchak. It appears that silencing of the (*a*) allele is the most likely explanation of our observations, making the homozygotic state the derived condition.

At *Pnp-A*, either recombination or mutation is again the most likely source of the derived homozygotic (*bb*) condition in some individuals in the Papanino population. As with *sIdh-A*, multiple origin of *L. armeniaca* seems unlikely, since *L. valentini* possesses only the (*b*) allele and *L. mixta* possesses only the (*a*) allele (MacCulloch et al. 1995; R.W. Murphy, unpublished data).

As in this study, Uzzell and Darevsky (1975) found that all individuals of *L. armeniaca* were heterozygous at *Ck-C* and *Mpi-A* and homozygous at *Gpi-A*. The genetic variability in *L. armeniaca* was greater than that found in three other parthenogenetic species of *Lacerta* examined (*L. dahli*: mean heterozygosity (MHD) = 0.421–0.428, mean number of alleles per locus (MNA) = 1.43–1.46, percentage of loci that are polymorphic (PLP) = 42.86; *L. rostombekovi*: MHD = 0.324, MNA = 1.32, PLP = 32.35; *L. unisexualis*: MHD = 0.290, MNA = 1.29, PLP = 29.03; R.D. MacCulloch, unpublished data). *Lacerta armeniaca* also exhibited heterozygosity at more loci (16/35) than *L. dahli* (15/35), *L. rostombekovi* (11/34), or *L. unisexualis* (10/34). Dessauer and Cole (1986) found a mean heterozygosity (method of calculation not stated) of 0.33–0.40 and a MNA of 1.37–1.40 in diploid parthenogenetic lizards of the teiid genus *Cnemidophorus*. Thus the levels of variation in *L. armeniaca* are approximately equivalent to those of teiid lizards. Variation in this study was much less than that found by Moritz et al. (1989) in the parthenogenetic form of the gecko *Heteronotia binoei*, which has multiple hybrid origins.

Three clones were detected in the *L. armeniaca* sampled, although sample sizes at some collecting sites were small. Of the seven populations examined, five contained only the principal widespread clone. Of the two rare clones, one occurred only in the Papanino population and the other only at Kutchak. This pattern of one widespread clone and other more restricted clones is typical of parthenogenetic lizards of the genus *Cnemidophorus* (Parker et al. 1989).

The model of diversity in hybrid parthenogenetic *Cnemidophorus* (Parker et al. 1989) proposed that "rare clones differing at single gene loci should be sympatric

with a common ancestral clone of the same cluster and should be morphologically similar." This model appears to be true in *L. armeniaca* at Papanino. The two clones co-occur and all individuals are identifiable as *L. armeniaca* by established morphological criteria. However, a different situation may have occurred at Kutchak. Although the two specimens collected at Kutchak were both from a unique clone, this does not preclude the presence of the more widespread clone at this location. Both Kutchak individuals are readily identifiable as *L. armeniaca*.

Based on a survey of four loci (*Ck-C*, *Gpi-A*, *Mpi-A*, and haemoglobin), Uzzell and Darevsky (1975) found only a single clone in two populations of *L. armeniaca*. Uzzell and Darevsky (1975) did not examine either of the loci that were found to be variable in our study. The clonal diversity of *L. armeniaca* is low, approximately equal to that in *Cnemidophorus tesselatus* (Parker 1979), but not as low as that in *C. neomexicanus* (Parker and Selander 1984), *L. rostombekovi*, or *L. unisexualis* (R.D. MacCulloch, unpublished data).

According to Moritz et al. (1992), low diversity for both allozymes and mitochondrial DNA in parthenogenetic species suggests that the species' origin involved few parental individuals and occurred in a restricted area. *Lacerta armeniaca* exhibited low diversity in both mtDNA (Moritz et al. 1992) and allozymes (this study), so a restricted origin is possible, as was postulated for *C. neomexicanus* (Parker and Selander 1984).

Lacerta valentini, one of the parents of *L. armeniaca*, exhibits variation at some loci (*sAat-A*, *Cat-A*, *G6pdh-A*, *Pep-A*, *Pep-B*, *sSod-A*, and *Tpi-A*) that were invariant in *L. armeniaca* (MacCulloch et al. 1995). This supports the theory that few parental individuals were involved in the species' origin. For the other parent, *L. mixta*, data are available from only seven individuals, all from one population; none exhibits variation at loci that were found to be invariant in *L. armeniaca* (R.W. Murphy unpublished data).

Clonal diversity has also been found to be related to niche breadth and range size in parthenogenetic species (reviewed in Parker et al. 1989). *Lacerta armeniaca* has a large contiguous range that overlaps the ranges of its parental species only slightly (Darevsky 1967; Darevsky et al. 1989). The species appears to be able to occupy more xeric habitats than its parents (Uzzell and Darevsky 1975). Many climatic and vegetative changes have occurred postglacially in the Caucasus region, providing a plethora of lizard microhabitats. Further examination of the species' ecology as well as the genetic makeup of other populations is required to better understand these relationships in *L. armeniaca*.

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Appendix

Specimens examined

Lacerta armeniaca: ROM 24138–24140, 24145, 24146, 24148–24149, 24153, 24155, 24158, 24159, 24163, 24165–24169, 24171–24173, 24176–24179, 24181, 24183, 24187, 24188, Armenia, Ankavan, valley of Marmaric River, 40°38′15″N, 44°32′54″E, elevation 1872 m; ROM 24109–24111, 24114, 24116–24118, 24120–24124, 24126, 24128, 24196, 24197, 24199, 24200, 24202, 24206, 24209, 24217, 24219, 24221, 24223, 24227, 24230, Armenia, Papanino, roadside dump, 40°44′39″N, 44°49′14″E, elevation 1212 m; ROM 24129–24134, Armenia, Sevan, hillside above cemetary, 40°30′58″N, 44°56′26″E, elevation 2100 m; ROM 24191–24194, Armenia, Stepanavan, valley of Dzozaget River, 41°01′15″N, 44°22′54″E, elevation 1363 m; ROM 24752–24758, Armenia, Sevan Pass, "Darevsky's Wall", 40°41′12″N, 44°51′20″E, elevation 1760 m; ROM 24978–24979, Armenia, Aragats mountain, Kutchak, 40°18′N, 43°40′E; ROM 24980, Armenia, Tumanyan, valley of Debet River, 40°59′N, 44°38′E.

Note: Co-ordinates measured to seconds were measured using a Global Positioning System; co-ordinates measured to minutes were estimated from maps.