Clonal variation in the Caucasian rock lizard *Lacerta armeniaca* and its origin

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Abstract. Clonal variation in *Lacerta armeniaca* was investigated using allozyme electrophoresis and morphology. Among the 35 allozyme loci examined, three were variable, which divided *L. armeniaca* into four clones. One rare clone of *L. armeniaca* made up the majority of two populations. This contrasts to rare clones in other parthenogenetic Caucasian rock lizards which typically consist of only one or two individuals. Another rare clone, which showed a striking colouration difference, had different allelic composition at two loci. Although mutation is a possible explanation of the origin of the clonal variation, the alternative, multiple origin, is equally likely.

Introduction

The unisexual Caucasian rock lizards (genus *Lacerta*) arose from interspecific hybridization of bisexual species and reproduce by way of cloning (Darevsky, 1967, 1992; Darevsky et al., 1985). They are true parthenogenetic species. Seven parthenogenetic species of Caucasian rock lizard have been described to date. The genetic variation within these lizard species has recently been subjected to detailed investigations by means of allozyme electrophoresis and mtDNA study (Moritz et al., 1992; MacCulloch et al., 1995b, 1997; Murphy et al., 1997; Fu et al., 1998; Fu, 1999). All but one species showed a similar pattern, in that each species consists of one common, widespread clone and a few rare clones. Each of the rare clones includes only one or two individuals although a large number of individuals were surveyed, and differs from the common clone at one allozyme locus. The only exception to this pattern is *L. armeniaca*.

Lacerta armeniaca originated from interspecific hybridization of *L. valentini* and *L. mixta* (Darevsky, 1992; MacCulloch et al., 1995b). It is widely distributed in central Armenia and southern Georgia. Allozymes in this species have been examined by Uzzell and Darevsky (1975) and MacCulloch et al. (1995b). Uzzell and Darevsky (1975) examined five loci in 11 specimens from two populations, which confirmed the hybrid origin of *L. armeniaca* and the identity of its parental species. All 11 specimens belonged to a single clone. MacCulloch et al. (1995b) conducted a more comprehensive examination, including 35 loci and 75 specimens from seven populations. The results revealed one widespread clone with two rare clones. However, in contrast to the pattern in other parthenogenetic rock lizards, one of the rare clones constituted the majority of one population (Papanino, Armenia, 19 out of 27 specimens) and the other appeared to constitute an entire population (Kutchak, Armenia, $n = 2$).

MacCulloch et al. (1995b) attributed the clonal variation in *L. armeniaca* to mutation, which is concordant with conclusions derived from other studies of parthenogenetic Caucasian rock lizards (Murphy et al., 1997; Fu et al., 1998), as well as other parthenogenetic lizards (see Moritz et al., 1989a for review). Mutations in *L. armeniaca* have been detected not only by allozyme data, but by morphological data as well. Darevsky (1992) first described some differences in colouration among specimens of *L. armeniaca* and concluded that the variation was the result of mutation.

An understanding of clonal variation is a necessary precursor to inferring the mode of origin of parthenogenetic species. Recent fieldwork yielded a substantial additional number of specimens from three locations, including some specimens with the colour variation described by Darevsky (1992). With a significant increase in sample size and new evidence, the clonal variation and mode of origin of *L. armeniaca* can be further examined.

Material and methods

A total of 42 specimens from three locations was collected and examined in this study. These included 20 additional specimens from Kutchak (Armenia, Aragatz mountain, 40°18′N, 043°40′E; ROM 26483-26502), where MacCulloch et al. (1995b) found that the entire sample belonged to an otherwise rare clone; 20 specimens from Bakuriani (Georgia, 41°40′N, 043°30′E; ROM 26503-26522) representing the most northerly extremity of the distribution and isolated from the main relatively contiguous range of the species; two specimens from Ankavan (Armenia, valley of Marmaric River, 40°38′15″N, 044°32′54″E; ROM 26456, 26460), which display the different colour pattern.

Electrophoresis protocols and nomenclature of enzymes and loci follow Murphy et al. (1996) and MacCulloch et al. (1995b). Whenever possible, two buffer systems were used to maximize the possibility of revealing variation. Specific buffer systems were the same as in Fu et al. (1995), MacCulloch et al. (1995a) and Bobyn et al. (1996). All electrophoretic data were compared to those of MacCulloch et al. (1995b).

Results

A total of 28 enzyme systems encoded by 35 presumptive gene loci were resolved and recorded. Alleles were fixed in homozygous states at 17 loci: mAat-A, Ada-A, Cbp-1, Ck-A, Gda-A, ßGlur-A, ßGlus-A, Gpi-A, Gtdh-A, G6pdh-A, mIdh-A, Ldh-A, sMdh-A, Pgm-A, Pk-A, mSod-A, Tpi-A. Fifteen loci were heterozygous: sAat-A, sAcoh-A, mAcoh-A, Acp-B, Cat-A, Est-D, Gcdh-A, Ldh-B, mMdh-A, mMdhp-A, sMdhp-A, Mpi-A, Pep-A, Pep-B, sSod-A. Three loci, Pnp-A, sIdh-A and Ck-C, were variable.

At sIdh-A, other than the two individuals from Kutchak, MacCulloch et al. (1995b) reported fixed heterozygosity for all specimens examined, with the faster allele supposedly coming from *L. mixta* and the slower allele from *L. valentini*. However, better allozyme resolution in this study revealed that *L. mixta*, *L. valentini* and all but three specimens of *L. armeniaca* examined share a single allele at sIdh-A. Although subtle differences in the media (starch) may be responsible for the discrepancy, careful re-examination of allozyme results concluded that the earlier erroneous recording was caused by artifacts. The three variant specimens of *L. armeniaca* were from Kutchak; they were heterozygous, with one common allele and a faster allele which has not been found in either parental species (table 1).

Most specimens were heterozygous at Pnp-A. MacCulloch et al. (1995b) found that 19 (out of 27) specimens from Papanino were homozygous for the slower allele. This study revealed that a majority (16 out of 22) individuals from the Kutchak population appeared to be homozygous for the slower allele, similar to the Papanino population. Interestingly, none of the 16 specimens has the rare genotype (ab) at locus sIdh-A. The two colour-variant individuals from Ankavan displayed the homozygous state for the faster allele (table 1).

At Ck-C, the two colour-variant individuals from Ankavan exhibited homozygosity for the slower allele. All other specimens were heterozygous. The three variable loci divided *L. armeniaca* into four clones (table 1).

The colour-variants from Ankavan are very distinctive. They do not exhibit the bright colours and the reticulate dorsal pattern of "normal" *L. armeniaca*, but rather they were grayish in colour. However, the scale patterns are exactly the same as the "normal" specimens. Darevsky (1992) considered the variable specimens to be "mutants".

Discussion

Although the common pattern of one widespread clone with few rare clones remained, the clonal variation in *Lacerta armeniaca* is exceptional in several respects. First, allozyme variation occurs at three loci, which is more variation than observed in any other parthenogenetic species of *Lacerta*. Second, in two populations the rare clones make up the majority of the individuals, although the common clone covered most of the species' distribution. At Papanino, 19 out of 27 individuals, and at Kutchak, 16 of the 22 specimens examined belong to a rare clone (clone 1; table 1). In both cases the clone exhibited

Table 1. Genotypes and sample sizes (in brackets) for the variable loci in the populations of *Lacerta armeniaca*. Lower case letters designate presumed alleles with "a" representing the fastest electromorph. Bold face letters indicate genotypes of rare clones. Pnp = Purine-nucleoside phosphorylase, EC 2.4.2.1; Idh = L-Isocitrate dehydrogenase, EC 1.1.1.42; Ck = Creatine kinase, EC 2.7.3.2.

	Papanino[1]		Sevan etc.[1]	Bakuriani	Kutchak[2]			Ankavan[2]		*mixta*[3]	*valentini*[4]
Pnp-A	**bb**(19)	ab(8)	ab(18)	ab(20)	ab(3)	ab(3)	**bb**(16)	aa(2)	ab(28)	aa(17)	bb(94)bc(1)
sIdh-A	bb(19)	bb(8)	bb(18)	bb(20)	bb(3)	**ab**(3)	bb(16)	bb(2)	bb(28)	bb(17)	bb(95)
Ck-C	ab(19)	ab(8)	ab(18)	ab(20)	ab(3)	ab(3)	ab(16)	**bb**(2)	ab(28)	aa(17)	bb(95)
	clone 1	clone 2				clone 3	clone 1	clone 4	clone 2	parental species	

[1] Data from MacCulloch et al. (1995b); Sevan etc. includes Sevan, Sevan Pass, Stepanavan, and Tumanyan; [2] Data of MacCulloch et al. (1995b) included; [3] Unpublished data; [4] Data from MacCulloch et al. (1995a).

homozygosity at Pnp-A. Third, the Ankavan colour-variants differ from the common clone at two loci, while rare clones of other parthenogens of *Lacerta* differ at only one locus.

Morphological variations were also observed. Parker et al. (1989) suggested that rare clones should be morphologically similar to the common clone. However, in *L. armeniaca*, the Ankavan population exhibited striking variation in colouration, which is clearly heritable from our observation. *Lacerta dahli* also showed apparent heritable morphological differences (Murphy et al., 1997). However, the allozyme analysis failed to detect any difference between the two morphs in *L. dahli*.

Where do these variations come from? The possible sources of genetic variation in parthenogenetic species are multiple origin, mutation and/or recombination (Parker and Selander, 1976). The observed allozyme variations in parthenogenetic Caucasian rock lizards have been attributed to mutation or recombination for two reasons. First, the numbers of individuals belonging to the rare clones are small; second, several new alleles, which had not been detected in the parental species, were present in the rare clones (MacCulloch et al., 1995b; Murphy et al., 1997; Fu et al., 1998). However, the variation in *L. armeniaca* is an exception to this rule, and gives rise to the question of whether these variations may have originated from multiple origins.

The "rare" clone that makes up the majority of the Kutchak and Papanino populations may have an independent origin. Geographically, the Kutchak and Papanino populations, which contain "successful" (i.e., numerous) rare clones, are located at the periphery of the main distribution. The dominance in number at two disjunct populations and the peripheral distribution to the common clone suggest multiple origins (Parker et al., 1989). The fact that *L. armeniaca* shares identical or extremely similar mtDNA with its maternal parent (*L. mixta*) further reinforced this possibility (Fu, 1999). The lack of mtDNA variation implies very recent origin. The contrast between the young age and substantial allozyme variation makes the multiple origins scenario more likely than rapid accumulation of mutations.

The allozyme variation pattern cannot reject the possibility that the observed clonal variations in *L. armeniaca* result from multiple origin. Multiple hybridization events leading to multiple clones (or species) have been hypothesized in other lizards (*Cnemidophorus* and *Heteronotia*; see Moritz et al., 1989a), as well as Caucasian rock lizards (Fu, 1999). Patterns of allozyme variation among clones of multiple origins varied from massive variation (*Heteronotia binoei*; Moritz et al., 1989b) to medium (*Cnemidophorus tesselatus*; Parker and Selander, 1976), and to slight (*L. unisexualis* and *L. uzzelli*; Fu et al., unpubl.). Thus, the patterns of allozyme variation in *L. armeniaca* do not contradict the possibility of multiple origins. Murphy et al. (1997) pointed out that, if multiple origins occurred, the variation of allelic composition among clones should be a result of parental allelic polymorphism. However, the "rare" clone of Kutchak and Papanino appeared to be homozygous for the slower allele at sIdh-A, although one of the parental species, *L. mixta*, is fixed at the faster allele. A number of reasons could explain this apparent contradiction. For example, either *L. mixta* lost the slower allele or the slower allele has not been detected. The latter ex-

planaiton is more likely because of the small sample size of *L. mixta* ($n = 17$) used in our investigation.

Although multiple origins are a likely explanation, mutation cannot be ruled out. Mutation is the simplest explanation for some of the genetic variation in *L. armeniaca* (clones 3 and 4; table 1). The Ankavan variants are most likely of mutant origin. These variants were first discovered in the 1970's, although many thousands of specimens had previously been collected from the same location. The colour-variants are, therefore, most probably a newly formed clone; it is unlikely to have arisen from an independent hybridization between *L. valentini* and *L. mixta* because these two parental species are not sympatric currently. Three specimens from Kutchak showed an allele that has not been detected in the parental species. It likely originated from mutation as well, although the allele may be present but undetected in the parental species.

Murphy et al. (1997) hypothesized that new mutants arising from within parthenogenetic species are short-lived. This concept resulted from two observations: the small numbers of individuals of the rare clones, which were hypothesized as being of mutant origin, and the deficiencies (lack of enzyme activity) in four loci of the specimens with new alleles in *L. dahli*. This concept is concordant with the general genetic rule that most mutations are deleterious. However, the evidence from *L. armeniaca* exemplified an apparent advantageous mutation. The Ankavan "mutant" colour-variant was first discovered in the early 1970's. In 1977, the proportion of colour-variants in the population did not exceed 1 to 2%, but in the late 1980's, it made up about 20% of a collection of *L. armeniaca* from Ankavan (Darevsky, 1992). Our 1995 expedition found two colour-variants among 32 specimens. The rapidly increasing numbers of the colour variants suggest that the new mutant is well-adapted to its environment. If the rare clones at Kutchak and Papanino originated from mutation rather than multiple origins, their abundance also demonstrates the success of new mutants.

In summary, the allozyme variation in *L. armeniaca* possibly derives from multiple origins, although mutation cannot be ruled out. A well-adapted mutant could increase its numbers in natural populations quickly. The origin and evolution of unisexuality in Caucasian rock lizards are more complicated than we previously perceived.

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