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Letter to the Editor

The Parthenogenetic Rock Lizard *Lacerta unisexualis:* An Example of Limited Genetic Polymorphism

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Abstract. Protein electrophoresis of *Lacerta unisexualis* from three populations found that 21 of 36 allozyme loci were homozygous, while 14 expressed fixed heterozygotes and one locus was variable. Three clones were detected at the locus Cat-A. Two individuals represent two rare clones while all others form a common clone. Our favored explanation is the mutation of a preexisting common clone rather than multiple origins.

Key words: Allozyme electrophoresis — Clonal variation — *Lacerta unisexualis* — Parthenogenesis — Unisexuality

Introduction

The origin and evolution of parthenogenetic species have intrigued biologists for about half a century. These uniparental species arose by hybridization between biparental species and they clone themselves from generation to generation. Recent investigations have shown that most parthenogenetic species exhibit some degree of diversity in their genetic makeup. The possible sources of the variation are mutation, recombination, or multiple origin (Cole et al. 1988; Moritz et al. 1989b; Parker 1979). Moreover, the amount of variation is correlated with the number of parental individuals involved in the hybridization and the area size of origin (Moritz 1991; Moritz et al. 1989a). The ecological parameters, distribution, and age of parthenogenetic species also contribute to the diversity (Dessauer and Cole 1989; Parker et al. 1989).

Lacerta unisexualis is one of the seven parthenogenetic species of the Caucasian rock lizards, genus *Lacerta,* subgenus *Archaeolacerta.* It originated from the hybridization of *L. valentini* and *L. raddei* (including *nairensis*) (Moritz et al. 1992; Uzzell and Darevsky 1975). Most of its distribution is currently fragmented into isolated small plots in central Armenia and eastern Turkey. The two parent species are not closely related to each other but rather belong to separate clades within the subgenus *Archaeolacerta* (Murphy et al. 1996a). *L. unisexualis* has also been shown to be a parent of both diploid and triploid hybrids (Darevsky and Danielyan 1968; Darevsky et al. 1989; Kupriyanova 1989). Examination of allozyme variability may help to clarify relationships among parthenogenetic species and their parents and to illuminate the processes whereby uniparental species have originated. Herein, we report the result of our study of genetic variation in *L. unisexualis.*

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Materials and Methods

Specimens of *L. unisexualis* were collected in central Armenia: Valley of the Marmaric River at Ankavan ($N = 27$), Aragatz Mountain near Kutchak ($N = 7$), and Nozaduz on Lake Sevan ($N = 23$). Voucher specimens are deposited in the collection of the Royal Ontario Museum (ROM). Museum catalogue numbers and locations are listed in Appendix 1.

Specimens were injected with an overdose of sodium pentobarbital and dissected immediately following euthanasia. Liver, heart, and tail muscle were removed and frozen in liquid nitrogen. Some specimens were frozen whole and dissected later. Upon arrival at the laboratory, the tissues were transferred to and stored in a −80°C freezer.

Enzymes were separated by horizontal gel electrophoresis on 11% starch gels. All electrophoretic procedures, protocols and nomenclature for loci, alleles, and enzyme systems follow Murphy et al. (1996b). Wherever possible, loci were resolved on two buffer systems to minimize the hidden variation. Specific buffer systems used for the locus products are as in Fu et al. (1995), MacCulloch et al. (1995a), and Bobyn et al. (1996).

Results of electrophoresis were analyzed using BIOSYS-1 release 1.7 (Swofford and Selander 1989). Mean heterozygosity by direct count (MHD), mean number of alleles per locus (MNA), and percentage of loci polymorphic (PLP) were used for evaluating genetic polymorphism. Alleles were also compared between *L. unisexualis* and its supposed parents.

Results

Twenty-eight enzyme systems encoded by 36 presumptive loci were resolved and recorded. Among the 36 loci, all individuals examined are homozygous for 21 loci including mAat-A, mAcoh-A, Ada-A, Cbp-1, Ck-A, Est-D, βGa-A, Gda-A, βGlur-A, βGlus-A, Gtdh-A, G6pdh-A, mIdh-A, sIdh-A, Ldh-A, mMdh-A, sMdh-A, Pep-B, Pgm-A, Pk-A, Tpi-A. All individuals exhibit heterozygosity in the following 14 loci: sAat-A, sAcoh-A, Acp-B, Ck-C, Gpi-A, Gpi-B, Ldh-B, mMdhp-A, sMdhp-A, Mpi-A, Pep-A, Pnp-A, mSod-A, sSod-A.

Variation occurs at the locus Cat-A. Two alleles are present at the locus. All individuals in the Ankavan and Nozaduz populations and all but two from Kutchak are heterozygous. One individual from Kutchak is homozygous for the slow allele, and one is homozygous for the fast allele.

Levels of genetic polymorphism from BIOSYS-1 are summarized in Table 1.

Table 1. Summary of genetic variability coefficients for the three populations of *Lacerta unisexualis*[a]

	Ankavan	Kutchak	Nozaduz
n	27	7	23
C_n	1	3	1
MHD	0.417 ± 0.083	0.409 ± 0.082	0.417 ± 0.083
MNA	1.42 ± 0.080	1.42 ± 0.080	1.42 ± 0.080
PLP	41.67	41.67	41.67

[a] n = sample size; C_n = number of clones. Standard errors are in parentheses; MHD = mean heterozygosity by direct count; MNA = mean number of alleles per locus; PLP = percentage of loci polymorphic (0.95 criterion)

Discussion

Our unpublished data confirm the hybrid origin of *L. unisexualis.* In all cases the alleles found in *L. unisexualis* were as expected for a hybrid between its supposed parents, *L. valentini* and *L. raddei* (including *nairensis*) (see Murphy et al. 1996a).

Fixed heterozygosity at some loci is expected in parthenogenetic species. Previous work by Uzzell and Darevsky (1975) showed that all of the *L. unisexualis* examined were heterozygous for the loci CK-C, GPI, and MPI. We found 14 loci with fixed heterozygosity. As suspected, the low variability in *L. unisexualis* is comparable to that found in *L. armeniaca* (MHD = 0.437–0.457, MNA = 1.46, PLP = 45.71; MacCulloch et al, 1995b), *L. rostombekovi* (MHD = 0.424, MNA = 1.42, PLP = 42.42; MacCulloch et al. 1997), and *L. dahli* (MHD = 0.392–0.400, MNA = 1.40–1.43, PLP = 40.00; Murphy et al., in press). Dessauer and Cole (1986) found mean heterozygosity (method of calculation not stated) of 0.33–0.40 and MNA of 1.37–1.40 in diploid parthenogenetic species of *Cnemidophorus.* Darevsky and Danielyan (1979) found that 96% of all skin graft trials were accepted among individuals from five populations of *L. unisexualis* and concluded that little variation existed.

Our populations of *L. unisexualis* contain three clones indicating a low level of diversity similar to that found other unisexual lacertids (MacCulloch et al. 1995b, 1997; Murphy et al. in press) and in *Cnemidophorus neomexicanus* (Parker and Selander 1984). The variation is much less than that found by Moritz et al. (1989a) in *Heteronotia binoei,* which probably originated from multiple hybridization events. Uzzell and Darevsky (1975) found only a single clone among two populations of *L. unisexualis;* Cat-A was not one of the loci analyzed in their study. There was no overlap of populations examined between our study and theirs.

Although most parthenogenetic species contain multiple clones (Parker 1979), the origin of genetic variation is often obscure. Possible origins are mutation, recombination, or multiple hybridization events (Moritz et al. 1992). The two individuals of *L. unisexualis* which differed from the others at the Cat-A locus were homozygous for alleles which occurred in the heterozygotic state in all other specimens examined. Comparisons with the parental species of *L. unisexualis* found that all *L. valentini* examined ($N = 95$) had only the fast allele at Cat-A while all *L. raddei* (incl. *L. nairensis*) ($N = 195$) had the slow allele plus another, faster than that found in *L. valentini* or *L. unisexualis* (Bobyn et al. 1996; MacCulloch et al. 1995a; Murphy et al. 1996a). The expected state for the hybrid parthenogenetic form *L. unisexualis*

would therefore be heterozygous. Multiple hybridization is unlikely as an explanation for the observed variation. Furthermore, given that the origin of unisexual species is an extremely rare event and highly constrained by phylogenetic, genetic, and other factors (Murphy et al. 1996a; Moritz et al. 1989a), there is little evidence supporting the possibility of multiple origins. Either recombination or mutation appears to be the most likely cause of the homozygous condition, as hypothesized for other parthenogenetic species of *Lacerta* (MacCulloch et al. 1995b; Murphy et al. in press). In the case of mutation, this could reflect either the silencing of one allele or loss of the gene itself.

Lacerta valentini and *L. raddei* exhibit considerable allozyme variation among populations (MacCulloch et al. 1995a; Bobyn et al. 1996) while the specimens of *unisexualis* examined herein are almost identical. Moritz et al. (1992) proposed that low allozyme diversity would imply that few individuals of the parental species were involved in the origin of the parthenogenetic hybrid, as appears to be the case in *Cnemidophorus neomexicanus* (Parker and Selander 1984). In addition, there are a few alleles which occur in the parental species that do not appear in *L. unisexualis.* The evidence suggests that the origin of *L. unisexualis* is locally and numerically restricted. Moritz et al. (1992) draw the same conclusion based on mitochondrial DNA fragment analysis. In their study, no variation was detected among four populations of *L. unisexualis* whereas variation existed among populations of the maternal bisexual species.

Although it appears that *L. unisexualis* originated from the hybridization of few parental individuals and only once, multiple origin cannot be completely excluded until other large populations in Turkey are examined. Further investigation of Turkish populations is desirable.

According to Uzzell and Darevsky (1975), *L. nairensis*, not *L. raddei*, is the maternal parent of *L. unisexualis.* Moritz et al. (1992), using mtDNA data, were unable to distinguish which of the two taxa was the parent. Examination of allozymes at 39 loci in 195 individuals from 10 populations failed to distinguish between *L. nairensis* and *L. raddei* (Bobyn et al. 1996), and the two taxa are considered to be conspecific for the purpose of this study. However, Fu et al. (in press) using DNA sequence data found that *L. nairensis* is phylogenetically closer to *L. saxicola* than to *L. raddei.* Accepting *L. saxicola* as a species makes *L. nairensis* a valid species. Further study is required to identify the maternal parents of *L. unisexualis.*

Clonal diversity is likely correlated with the age of the species (Dessauer and Cole 1989). The low diversity in *L. unisexualis* indicates the recent origin of the species. Uzzell and Darevsky (1975) suggested *L. unisexualis* is at least 5,000 years old. Our allozyme data do not give an estimation of the species' age. More detailed DNA sequence data about both the parental species and unisexual species may be informative.

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 unisexualis* has a discontinuous range and limited overlap with the ranges of its parental species (Darevsky 1967; Uzzell and Darevsky 1975). According to the latter, this distribution consists of relict populations rather than unique origins. *L. unisexualis* also appears to be able to occupy habitats which sexual species do not (Darevsky et al. 1989). Since the Würm glacial period, the Caucasus have undergone many habitat changes; these both limit distributions and provide new microhabitats for lizards. Further study of the ecology as well as the genetic makeup of other populations is required to better understand questions of genetic and ecological relationships of *L. unisexualis.*

Appendix 1: Specimens Examined

Lacerta unisexualis: ROM 24232–24258, Armenia, Ankavan, valley of Marmaric River, 40°38′ 15″ N, 044°32′ 54″ E; ROM 24985, 26772-26777, Armenia, Aragatz mountain, Kutchak, 40°18′ N, 043°40′ E. ROM 26799–26821, Armenia, Nozaduz, 40°30′ N, 044°20′ E.

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