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Old age, multiple formations or genetic plasticity? Clonal diversity in the uniparental Caucasian rock lizard, *Lacerta dahli*

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Abstract

Allozyme variation at 35 gene loci is investigated in 161 specimens of the uniparental Caucasian lizard *Lacerta dahli* from several locations in Armenia and Georgia. All individuals are heterozygotic at 12 loci, and homozygotic at 21 loci. Variation at two loci results in five uniparental clones. One clone is widespread whereas four are geographically restricted and are represented by only one or two individuals. Because successful formation of uniparental clones is rare, and because the biparental species forming them are now allopatric, the most probable explanation for the origin of the observed clonal diversity is either mutation or recombination within the common clone. The rare clones have lower levels of enzyme activity at four loci, suggesting that these organisms may be genetically deficient. Although the evidence points to change in a pre-existing clone, the possibility of multiple origins cannot be ruled out.

Introduction

The Caucasian lacertid lizards of the *saxicola*-complex include both biparental and uniparental species. Among these lizards, 12 biparental species, including many subspecies, and seven uniparental species have been described (Darevsky, Kupriyanova & Uzzell, 1985; Murphy et al., 1996a; Schmidtler, Eiselt & Darevsky, 1994). All uniparental species arose by hybridization between individuals of two sexually reproducing species. For *L. dahli*, *L. portschinskii* is the paternal parent (Uzzell & Darevsky, 1975; Murphy, Darevsky, Kupriyanova, MacCulloch & Fu, unpublished) and *L. mixta* has been confirmed as the maternal parent (Mortiz et al., 1992; Murphy, Darevsky, Kupriyanova, MacCulloch & Fu, unpublished). These two biparental species are not sister taxa but rather belong to different clades in the *saxicola*-complex (Darevsky, Kupriyanova & Uzzell, 1985; Murphy et al., 1996a; Fu, Murphy & Darevsky, in press; Murphy, Darevsky, Kupriyanova, MacCulloch & Fu, unpublished).

Lacerta dahli, a uniparental species, occurs in the southern Caucasus Mountains of Armenia and Georgia; the distribution of this species and the biparental taxa giving rise to it are shown in Uzzell and Darevsky (1975) and Darevsky, Kupriyanova, and Uzzell (1985). Two color varieties are known (Darevsky, 1967): one clone has pale greenish-yellow ventral scales whereas the other has a bright yellow belly. Bright yellow-bellied females produce fewer eggs than the pale yellow-bellied females, and they appear to be less tolerant to desiccation (personal observation, Danielyan, Darevsky, Kupriyanova and Murphy). The two varieties occur sympatrically in a roadside trash dump at Papanino, Armenia.

The two widespread color varieties of *Lacerta dahli* suggest the possibility of a significant degree of clonal variation in this taxon compared to other uniparental species in the *L. saxicola* group. Our examination of allozyme variation in *L. dahli* revealed several clones,

but, unlike the color varieties, one clone is widespread and abundant, and the others rare and localized. Herein we report our findings and consider the possible origins of this clonal variation, and the relationships among the clones.

Materials and methods

Specimens of *L. dahli* were collected at three localities in central and northern Armenia, in the southern portion of the species' range; and at three localities in central Georgia in the northern part of the range (Appendix 1). Following accepted animal welfare protocols, specimens were injected with an overdose of sodium pentobarbitol and dissected immediately following euthanasia. Liver, heart, and skeletal muscle tissues were removed and frozen in liquid nitrogen and subsequently stored at $-80°$C until used.

All electrophoretic methods and allelic nomenclature are those used by Murphy et al. (1996b). Our analysis utilized 27 enzyme systems encoded by 35 gene loci. Wherever possible, gene products were resolved on two buffer systems to maximize the likelihood of detecting all variants. Specific buffer systems used for resolving gene products are given by Bobyn et al. (1996), Fu et al. (1995), and MacCulloch et al. (1995a). For parentage determination, the specimens were compared with 16 biparental species, including outgroup members (Murphy et al., 1996a).

Genetic polymorphism, including mean heterozygosity, number of alleles per locus, and percentage of loci exhibiting polymorphism, was estimated using BIOSYS-1 release 1.7 (Swofford & Selander, 1989).

Results

In all individuals, 21 of the 35 loci resolved were homozygous in all populations, including: mAat-A, sAcoh-A, Ada-A, Cbp-1, Ck-A, Ck-C, βGa-1, Gda-A, βGlur-A, βGlus-A, Gpi-A, Gtdh-A, G6pdh-A, mIdh-A, sIdh-A, Ldh-A, mMdh-A, sMdh-A, Pgm-A, Pk-A, Tpi-A. All individuals in all populations were heterozygous at the following 12 loci: sAat-A, mAcoh-A, Acp-B, Cat-A, Gcdh-A, Gpi-B, Ldh-B, sMdhp-A, Pep-A, Pep-B, mSod-A, and sSod-A.

Variation occurred in specimens from Armenia at two loci, Mpi-A and Pnp-A, resulting in five clones. Only 5 of 161 individuals evaluated differed from the common clone. At Mpi-A, all individuals except one were heterozygous; from Stepanavan, a single individual (of nine surveyed) expressed a single allele that was slower than either of the two alleles in all other individuals, including those of the parental species. At Pnp-A, all but four individuals were heterozygous. One individual from Tumanyan was homozygous for the faster of the two common alleles. A single individual each from Stepanavan and Papanino was homozygous for the slower of the two common alleles. And at Papanino, one individual was homozygous for a unique allele that was faster than either of the two common alleles. No individual differed from the common clone at more than one locus. In addition to genotypic differences, these five variant specimens also exhibited no enzyme activity at four other loci (Acp-B, Est-D, βGa-1, and Pep-A). Variation was not found in populations from Georgia.

For each of the six populations surveyed, estimates of mean heterozygosity (by direct count), mean number of alleles per locus, and percentage of loci polymorphic (0.95 criterion) are provided in Table 1. Comparisons with other uniparental *Lacerta* are given in Table 2.

Discussion

Because of the obvious morphological variation, we would predict similar variability in the genetic makeup of *L. dahli*, along with expected high levels of heterozygosity. Among uniparental reptiles, genetic variation can be correlated with the number of biparental individuals involved in the origins of the daughter uniparental species, the size of the area of origin (Moritz, 1991; Moritz et al., 1992), and the ecology, distribution, and age of uniparental taxa (Dessauer & Cole, 1989; Parker, Walker & Paulissen, 1989). Possible sources of this clonal variation are either mutation, recombination, or multiple origins (Cole, Dessauer & Barrowclough, 1988; Moritz et al., 1989; Parker, 1979).

Four relatively rare clones, each represented by one or two individuals, were detected in three sampled populations of *L. dahli*. Of the rare clones, one each occurred only in the Papanino, Stepanavan, and Tumanyan populations. The fourth rare clone was found at both Papanino and Stepanavan. This pattern of one widespread clone and other, more restricted clones is typical of both uniparental *L. armeniaca* (MacCulloch et al., 1995b) and *L. unisexualis* (Fu et al., in press). A similar pattern of distribution is also typi-

Table 1. Summary of genetic variability coefficients for the six populations of *Lacerta dahli.* n = sample size; C_n = number of clones. Standard errors are in parentheses

	Stepanavan	Papanino	Tumanyan	Kodjori	Kareli	Manglisi
n	9	86	24	11	16	15
C_n	3	3	2	1	1	1
MHD	0.392(.083)	0.399(.084)	0.399(.084)	0.400(.084)	0.400(.084)	0.400(.084)
MNA	1.40(.08)	1.43(.09)	1.40(.08)	1.40(.08)	1.40(.08)	1.40(.08)
PLP	40.00	40.00	40.00	40.00	40.00	40.00

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

Table 2. Comparison of allozyme variability in *Lacerta dahli* with variability in three other uniparental species

	L. dahli	*L. unisexualis*	*L. rostombekovi*	*L. armeniaca*
C_n	5	3	1	3
MHD	0.392–0.400	0.409–0.417	0.424	0.437–0.457
MNA	1.40–1.43	1.42	1.42	1.46
PLP	40.00	41.67	42.42	45.71

C_n = number of clones.
MHD = mean heterozygosity by direct count.
MNA = mean number of alleles per locus.
PLP = percentage of loci polymorphic.

cal of uniparental *Cnemidophorus* (Parker, Walker & Paulissen, 1989).

Similar to our study, Uzzell and Darevsky (1975) found that all individuals of *L. dahli* were heterozygous at Mpi-A and homozygous at Ck-C and Gpi-A. Unlike our study, they did not detect variation in Mpi-A. The discrepancy likely derives from their survey of 14 specimens from four localities compared to our evaluation of 161 specimens from six localities.

The genetic variability in *L. dahli* in our study (Table 1) was the least known relative to that found in other uniparental species of *Lacerta* investigated thus far (Table 2). These measures of variability reflect divergence between the biparental species and not variability within uniparental species, per se. In comparison, Dessauer and Cole (1986) found mean heterozygosity (method of calculation not stated) of 0.33–0.40 and mean numbers of alleles per locus of 1.37–1.40 in diploid uniparental species of the teiid genus *Cnemidophorus*.

With five clones, the clonal diversity of *L. dahli* is the greatest yet detected among uniparental *saxicola*-complex *Lacerta*. Three clones occur in *L. armenica* (MacCulloch et al., 1995b) and *L. unisexualis* (Fu et al., in press), and one in *L. rostombekovi* (MacCulloch et al., 1997). This level of variation is approximately equal to that in *Cnemidophorus tesselatus* (Parker, 1979), but greater than in *C. neomexicanus* (Parker & Selander, 1984). Variation in *L. dahli* is much less than that found by Moritz et al. (1989) in the uniparental taxa of geckos called *Heteronotia binoei*, which has multiple hybrid origins.

All specimens of *L. dahli* were homozygous for the allele sAcoh-A(b). Although this allele occurs in all three populations of *L. portschinskii* examined from Georgia (MacCulloch et al., in press), it appears to be absent in *L. portschinskii* from Armenia (MacCulloch et al., 1995a). This distribution suggests 1) that *L. dahli* arose in Georgia and dispersed southward into Armenia; 2) that *L. dahli* arose in Georgia but subsequently the (b) allele of sAcoh-A disappeared from Armenian populations; or 3) that the allele occurs in Armenia at a low frequency but has eluded our detection. The northern distribution of the other parent, *L. mixta*, supports the first scenario, whereas the significant genetic substructuring in other biparental species (Bobyn al., 1996) supports the third possibility; the second scenario is not testable with allozyme data, although sufficient DNA sequence data might be informative.

As expected in a uniparental species, all loci but two in *Lacerta dahli* exhibited fixation of alleles in a homozygous or heterozygous state. Possible origins of the observed variation at the two loci are recombination, mutation, multiple origin (Moritz et al., 1989; Parker, 1979), or gene silencing. At Pnp-A, three of the four variable individuals were homozygous for one of the two alleles possessed by all other *L. dahli*. This implies that either mutation or recombination is the likely cause of the difference. In the case of mutation, this would likely be the silencing of one allele. Although one individual each in the Papanino and Stepanavan populations exhibited the same unique combination of alleles, these likely represent independent losses of heterozygosity, rather than remnant members of a widespread clone. The fourth individual possessed a unique Pnp-A allele found neither in other *L. dahli* nor in either parental species (Murphy, Darevsky, Kupriyanova, MacCulloch & Fu, unpublished). A similar situation occurs in a single individual that possessed a unique allele at Mpi-A. There are two possible explanations for this: either 1) these alleles are present in the parental species but have not been detected, or 2) the individuals in question, or their clonal ancestors, underwent mutations at these loci. The first explanation requires multiple origins of clones.

Several lines of evidence suggest that the allozymic uniparental clones observed in *L. dahli* result from changes within the common clone rather than multiple origins. The formation of uniparental lineages is not a simple consequence of hybridization. Crosses involving biparental species involved in the formation of uniparental species have failed to produce uniparentals (Danielyan, 1981). Further, Murphy et al. (1996a) and Murphy, Darevsky, Kupriyanova, MacCulloch and Fu (unpublished) discovered more alleles in the parental species than were resolved in *L. dahli*. The male biparental species, *L. portschinskii*, exhibited variation at four loci that were invariant in *L. dahli* (MacCulloch et al., 1995a). For the maternal parent, *L. mixta*, allozyme data were available from only one population. However, four alleles encoded by three loci occurred in *L. mixta* but were absent in *L. dahli*. Thus, if clones were frequently formed, then we would anticipate finding a large number of clonal variants, both sympatrically, and across the range. Given the relatively low number of clones resolved and current allopatric occurrence of the parental species, it appears as though few biparental individuals were involved in the formation of *Lacerta dahli* and that the initial uniparental clone was formed only once.

Low diversity for both allozymes and mitochondrial DNA in uniparental species also suggests that the species' origin involved few parental individuals and that it occurred in a restricted area (Moritz et al., 1992), that is if the genotypes present match one of the parental genotypes. Otherwise, a population bottleneck could provide an alternative explanation. *Lacerta dahli*, which exhibits existing parental genotypes, exhibited low diversity in mtDNA (Moritz et al., 1992).

The few variant individuals have no discernable enzyme activity at four loci, so it is unlikely that they are members of persistent lineages. Rather, it seems probable that the mutations arose independently in the individuals examined. Is it likely that the loss of heterozygosity at a single locus would prevent the clonal descendants from increasing in number? Probably not. However, in these *L. dahli* clones, the corresponding reduced enzyme activity at four loci likely have a greater affect. Apparently, we are observing multi-gene effects, although the source remains to be identified. Even if such a great loss is not the case, reduced fitness, resulting from deficiency of a number of enzymes, is likely.

Because clonal diversity may also be a function of the species' age (Dessauer & Cole, 1989) the possibility exists that *L. dahli* may be older than other Caucasus uniparental *Lacerta*. However, given that the variant clones are restricted to only one or two specimens, and the likelihood that these are not long-lived successful clones, we do not believe that these particular clones of *L. dahli* are significantly older than the clones of other species. We predict that genetic surveys of uniparental species will turn up variant individuals such as these. These ephemeral variants likely have little effect on the evolution of the species.

The study of clonal diversity poses questions as to why rare clones are rare, and why their numbers do not increase. Among the uniparental *Lacerta* examined, the only apparent persistent lineage(s) other than the common clone occurs in *L. armeniaca*. In Papanino, 19 of 27 individuals belonged to a clone found only in that population; the remaining 8 individuals belonged to the principal, widespread clone (MacCulloch et al., 1995b). The variant individuals of *L. armeniaca* did not exhibit the reduced enzyme activity found in variant *L. dahli*.

We believe that *L. dahli* has at least two successful clones. In one form, the ventral scales are pale yellow, whereas another has bright yellow bellies. Bright

yellow-bellied females produce fewer eggs than the pale yellow-bellied females (Darevsky & Danielyan, unpublished data), and they appear to be less tolerant to desiccation (personal, unquantified observation). Our allozyme data failed to distinguish these two clones, but this is not surprising given that we have sampled from only a small fraction of the genome. Further research may reveal genetic markers that differentiate these two clones.

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Appendix

Specimens examined

Armenia, Papanino, 40° 44′ 39″ N, 044° 49′ 14″ E, ROM 24009–24026, ROM 24036–24105; Armenia, Stepanavan, 41° 01′ 15″ N, 044° 22′ 54″ E, ROM 24027–24035; Armenia, Tumanyan, 40° 59′ N, 044° 38′ E, ROM 24935–24957; Georgia, Kodjori, 41° 38′ 32″ N, 044° 41′ 02″ E, ROM 26525–26535; Georgia, Kareli, 42° 01′ N, 043° 52′ E, ROM 26538–26553; Georgia, Manglisi, 41° 43′ N, 044° 25′ E, ROM 26554–26569.