



## The Caucasian Rock Lizard *Lacerta rostombekovi*: a Monoclonal Parthenogenetic Vertebrate

ROSS D. MACCULLOCH,\* ‡ ROBERT W. MURPHY,\* LARISSA A. KUPRIYANOVA† and  
ILYA S. DAREVSKY†

\*Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, 100 Queen's Park, Toronto,  
Ontario M5S 2C6, Canada;

†Zoological Institute, Academy of Sciences, St. Petersburg 119034, Russia

**Key Word Index**—*Lacerta rostombekovi*; Lacertidae; rock lizard; parthenogenesis; unisexuality; clonal diversity; allozymes.

**Abstract**—Among vertebrates, true parthenogenesis (self-perpetuating all-female species) occurs only in reptiles; these species are of hybrid origin. To date, all diploid parthenogenetic reptiles examined exhibit some genetic diversity, resulting in the existence of more than one clone. The sole exception to this is the Caucasian rock lizard *Lacerta rostombekovi*, which appears to consist of only a single clone. © 1997 Elsevier Science Ltd. All rights reserved

### Introduction

Although well-documented, the phenomenon of parthenogenesis is not well understood. Questions dealing with the ecology, genetics, frequency of hybridization, genealogical constraints on parthenogenesis, and mechanisms for failed meiosis are paramount. Among vertebrates, true parthenogenesis has been detected only in squamate reptiles, and especially among lizards (Vrijenhoek *et al.*, 1989). All diploid parthenogenetic species examined have been shown to exhibit intraspecific genetic diversity; each 'species' consists of multiple clones, caused by either mutation, multiple origins or genetic recombination (Cole *et al.*, 1988; Parker, 1979a; Parker and Selander, 1984). Clonal diversity has been related to distribution, ecological parameters, and inferred age of parthenogenetic species (Dessauer and Cole, 1989; Parker, 1979b).

Because of their origin through hybridization, parthenogenetic species exhibit characteristics of F<sub>1</sub> hybrids, including a high level of fixed heterozygosity. In hybridogenesis there must be a balance between too much genetic divergence between parental species, which would result in developmental failure, and hybridization, permitting viable offspring and backcrossing. This has been termed the 'balance hypothesis' (Moritz *et al.*, 1989). The relationships among potential parental species require further study.

The genus *Lacerta* contains several parthenogenetic species, all of which were formed by hybridization between bisexual species; in fact, parthenogenesis in reptiles was first discovered in this genus (Darevsky, 1958). One of these unisexual species, *L. rostombekovi*, arose from the hybridization of a male *L. portschinskii* and a female *L. raddei* (Darevsky *et al.*, 1985; Moritz *et al.*, 1992; Murphy *et al.*, in press). The two parental

‡Corresponding author (ROSSM@ROM.ON.CA).

bisexual species are members of different clades in the genus *Lacerta* (Darevsky *et al.*, press; Murphy *et al.*, in press). As part of a study of population genetics of Caucasian *Lacerta*, we examined the genetic diversity in *L. rostrombekovi* and surprisingly identified only a single clone.

## Materials and Methods

The range of *Lacerta rostrombekovi* consists primarily of a large contiguous area north and east of Lake Sevan, Armenia. In addition, one disjunct population occurs northwest of Lake Sevan (Darevsky *et al.*, 1985), and a second isolated population, at the southeastern end of the lake, has recently been extirpated (Darevsky, unpublished observation). Specimens were collected from four localities; Papanino (40°44'N, 044°49'E), Gosh (40°45'N, 045°01'E), Agartchin (40°48'N, 044°46'E) and Spitak (40°51'N, 044°19'E). Although the first three locations are within the large contiguous area of the species' range, the mountainous terrain causes patchy distributions (Darevsky, 1967); populations of conspecific *Lacerta*, both bisexual and parthenogenetic, from within a contiguous range often exhibit genetic differentiation (Bobyn *et al.*, in press; MacCulloch *et al.*, 1995a; Moritz *et al.*, 1992). Therefore, we are confident that these three locations represent discrete populations. The fourth location, Spitak, occurs within the range of the remaining disjunct population of *L. rostrombekovi* (Darevsky *et al.*, 1985). A total of 65 specimens were collected from the four locations.

Specimens were euthanized with an overdose of sodium pentobarbital and dissected immediately, following approved animal welfare protocols. Liver, heart and skeletal muscle were dissected and frozen in liquid nitrogen. Voucher specimens are deposited in the herpetological collection of the Royal Ontario Museum (ROM).

Genetic diversity was assayed by protein electrophoresis following established methods (Murphy *et al.*, 1996). Our data included 28 enzyme systems encoded by 35 presumptive gene loci; standard locus names and abbreviations (Murphy *et al.*, 1996) are used. Specific buffer systems used for the separation of loci are the same as those used in other studies of Caucasian *Lacerta* (Bobyn *et al.*, in press; Fu *et al.*, 1995; MacCulloch *et al.*, 1995b). Alleles present in *L. rostrombekovi* were compared to those in its supposed parental species, *L. portschinskii* and *L. raddei*. The allozyme data were analysed using BIOSYS-1 release 1.7 (Swofford and Selander, 1989) for heterozygosity, number of alleles per locus and percent of polymorphic loci.

## Results

All individuals were genetically identical at all loci examined. All individuals examined were homozygous at 20 of the 35 loci resolved: mitochondrial aconitase hydratase (mAcoH-A; EC 4.2.1.3), adenosine deaminase (Ada-A; EC 3.5.4.4), calcium-binding protein (Cbp-1; nonspecific), creatine kinase 'A' (Ck-A; EC 2.7.3.2), esterase-D (Est-D; EC 3.1.1.-), guanine deaminase (Gda-A; EC 3.5.4.3),  $\beta$ -glucuronidase ( $\beta$ Glur-A; EC 3.2.1.31),  $\beta$ -glucosidase ( $\beta$ Glus-A; EC 3.2.1.21), glutamate dehydrogenase (Gtdh-A; EC 1.4.1.2), glucose-6-phosphate dehydrogenase (G6pdh-A; EC 1.1.1.49), n-acetyl- $\beta$ -glucosaminidase ( $\beta$ Ga-1; EC 3.2.1.30), supernatant isocitrate dehydrogenase (sldh-A; EC 1.1.1.42), mitochondrial isocitrate dehydrogenase (mldh-A; EC 1.1.1.42), l-lactate dehydrogenase 'A' (Ldh-A; EC 1.1.1.27), mitochondrial malate dehydrogenase (mMdh-A; EC 1.1.1.37), peptidase-B (leucyl-glycyl-leucine) (Pep-B; EC 3.4.-.-), phosphoglucomutase (Pgm-A; EC 5.4.2.2), pyruvate kinase (Pk-A; EC 2.7.1.40), mitochondrial superoxide dismutase (mSod-A; EC 1.15.1.1) and triose-phosphate isomerase (Tpi-A; EC 5.3.1.1). All individuals were heterozygous at the remaining 15 loci: supernatant aspartate aminotransferase (sAat-A; EC 2.6.1.1), supernatant aconitase hydratase (sAcoH-A; EC 4.2.1.3), acid phosphatase (Acp-B; EC 3.1.3.2), catalase (Cat-A; EC 1.11.1.6), creatine kinase 'C' (Ck-C; EC 2.7.3.2), glucose dehydrogenase (Gcdh-A; EC 1.1.1.118), glucose-6-phosphate isomerase 'A' (Gpi-A; EC 5.3.1.9), glucose-6-phosphate isomerase 'B' (Gpi-B; EC 5.3.1.9), l-lactate dehydrogenase 'B' (Ldh-B; EC 1.1.1.27), mitochondrial malate dehydrogenase (NADP+) (mMdhp-A; EC 1.1.1.40), supernatant malate dehydrogenase (NADP+) (sMdhp-A; EC 1.1.1.40), mannose-6-phosphate isomerase (Mpi-A; EC 5.3.1.8), peptidase-A (glycyl-leucine) (Pep-A; EC

3.4.-.), purine-nucleoside phosphorylase (Pnp-A; EC 2.4.2.1) and supernatant superoxide dismutase (sSod-A; EC 1.15.1.1).

All alleles present in *L. rostombekovi* were also found in the supposed parental species. At those loci for which *L. rostombekovi* was homozygous, both parents also possessed the same allele; at those loci for which *L. rostombekovi* was heterozygous, each parent possessed only one of the two alleles.

The results of analysis using BIOSYS-1 are as follows: mean heterozygosity by direct count (MHD) =  $0.424 \pm 0.087$ ; mean number of alleles per locus (MNA) =  $1.42 \pm 0.08$ ; percent of loci polymorphic = 42.42%.

## Discussion

The coefficients of genetic variability found by BIOSYS-1 in *L. rostombekovi* were comparable to those found in other parthenogenetic *Lacerta* so far examined: *L. armeniaca* MHD = 0.437–0.457, MNA = 1.46, PLP = 45.71; *L. dahli* MHD = 0.392–0.400, MNA = 1.40–1.43, PLP = 40.00; *L. unisexualis* MHD = 0.409–0.417, MNA = 1.42, PLP = 41.67 (MacCulloch *et al.*, 1995a; unpublished observations). Each of these three species contains more than one clone, as demonstrated by ranges in the coefficient MHD.

The genetic homogeneity found among the populations of *L. rostombekovi* is not reflected in morphology; a morphological examination of specimens from three populations found considerable variation (Darevsky *et al.*, 1985). A model of genetic and morphological diversity in hybrid parthenogenetic species predicts that morphologically distinguishable clones will also be genetically distinguishable (Parker *et al.*, 1989). It appears that *Lacerta rostombekovi* does not conform to this prediction. A similar situation has been found in the parthenogenetic teiid lizard *Cnemidophorus tesselatus* (Parker, 1979a; Parker *et al.*, 1989), in which some pattern classes are not distinguishable genetically. Whether this reflects undetected genetic variability or abiotic effects remains to be determined.

All other diploid parthenogenetic reptiles so far examined contain more than one clone. The three other parthenogenetic *Lacerta* (*L. armeniaca*, *L. dahli* and *L. unisexualis*) from the Caucasus Mountains region which have so far been examined are multiclonal (MacCulloch *et al.*, 1995a; unpublished observations). In the family Teiidae, all diploid parthenogenetic species contain more than one clone (Parker and Selander, 1984; Dessauer and Cole, 1989). The diploid geckos *Nactus pelagicus* and *Lepidodactylus lugubris* contain several clones (Donnellan and Moritz, 1995; Pasteur *et al.*, 1987). Two clones were detected in the parthenogenetic xantusiid lizard *Lepidophyma flavimaculatum* (Bezy and Sites, 1987). Monoclonality appears to be more common among triploid parthenogenetic reptile species (Darevsky *et al.*, 1984; Dessauer and Cole, 1989; Wynn *et al.*, 1987), although this may be a function of the number of populations examined. An exception to this occurs in *Lepidodactylus lugubris*, where polyclony in triploids exceeds that in diploids. Although triploid individuals have been found among the Lacertidae, they are almost always sterile (Darevsky *et al.*, 1989); no triploid lineages have been detected.

The parental species of *L. rostombekovi*, *L. portschinskii* and *L. raddei*, exhibit considerable allozyme variation (MacCulloch *et al.*, 1995b; Bobyn *et al.*, in press). However, all 65 *L. rostombekovi* examined in this study are identical. Mitochondrial DNA from *L. rostombekovi* resembles that of only one population of its maternal parent, *L. raddei*

(Moritz *et al.*, 1992). *Lacerta rostombekovi* also possesses a unique submetacentric chromosome, one not found in any bisexual species of *Lacerta* in the region (Darevsky *et al.*, 1972). These data suggest a locally and numerically restricted origin for *L. rostombekovi*. Further, it has been proposed that low clonal diversity in a parthenogenetic species could indicate a recent origin for that species (Dessauer and Cole, 1989). Parthenogenetic *Lacerta* may have arisen in association with climatic and concomitant habitat changes during or following the Würm glaciations, approximately 10,000 ybp (Uzzell and Darevsky, 1975). If the presence of more than one clone is related to the age of a parthenogenetic species (Dessauer and Cole, 1989), then *L. rostombekovi* may have arisen even more recently.

Although the existence of multiple clones can only be disproved by examination of every individual of a species, equivalent samples from four populations have been sufficient to demonstrate clonal diversity in all diploid parthenogenetic species so far studied. In other parthenogenetic *Lacerta*, disjunct populations typically contain clonal variants (MacCulloch *et al.*, 1995a; unpublished observations), whereas in this study even the disjunct Spitak population did not differ from the other populations. Since all individuals of *L. rostombekovi* examined possess identical alleles at each locus, as well as the unique submetacentric chromosome (Darevsky *et al.*, 1972), it appears that only a single clone of this species exists.

**Acknowledgements**—Import permits for frozen tissues and preserved specimens were issued by Agriculture Canada and all collecting and specimen euthanasia was performed under approved animal protocols. This study was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada grant A3148, and by the generous assistance of the ROM Sciences Fieldwork Fund, the ROM Future Fund, the ROM Foundation, and especially the Department of ROM Volunteers to R. W. Murphy, and by the Zoological Institute of the Russian Academy of Sciences, the Russian Scientific and Technical Program "Priority Trends in Genetics", the Russian Foundation for Basic Science and the International Science Foundation (No. J3Y100) to I. S. Darevsky and L. A. Kupriyanova. Laboratory work was carried out in the Laboratory of Molecular Systematics of the ROM.

For assistance with field work and providing specimens, we are grateful to T. Morales-Sokolova, E. Roitberg, E. Yauruyan, F. Danielyan, M. Bakradze, V. Negmedzanov, D. Tarkhnishvili, I. Serbinova, B. Tuniyev, and especially N. Orlov and A. Agasian. Inestimable laboratory assistance was provided by D. Upton and M. Boby. Air France and KLM Airlines significantly contributed to our efforts by providing free excess baggage during international travels. This is contribution number 44 from the Centre for Biodiversity and Conservation Biology of the Royal Ontario Museum.

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