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Limited genetic variation in *Lacerta mixta* and its parthenogenetic daughter species: evidence from cytochrome *b* and ATPase 6 gene DNA sequences

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Abstract

Little mtDNA variation was observed among populations of the bisexual Caucasian rock lizard *Lacerta mixta* and unisexual *L. dahli* and *L. armeniaca*. Three haplotypes were detected in *L. mixta* and the maximum pairwise difference among the samples was 0.67%. No intra- and interspecific variation was found among populations of either *L. armeniaca* or *L. dahli*. Moreover, both unisexual species were identical to one of the three haplotypes of *L. mixta*. The limited variation in *L. mixta* is likely the result of bottleneck effect, although the small sample size may also be responsible. The lack of variation in the unisexuals was attributed to the restricted variation among the maternal parents, limited involvement of females in the hybridization, and recent origin.

Introduction

Parthenogenesis in Caucasian rock lizards (genus *Lacerta*) originated from interspecific hybridization of bisexual species (Darevsky et al., 1985; Darevsky, 1992). Two bisexual species, *Lacerta raddei* and *L. mixta*, have been identified as the maternal parents of the seven unisexual species (Darevsky et al., 1985; Schmidtler et al., 1994). *Lacerta mixta* is the maternal parent of two unisexual species, *L. armeniaca*, and *L. dahli* (Darevsky, 1992; Moritz et al., 1992; MacCulloch et al., 1995; Murphy et al., 1997). Both *Lacerta armeniaca* and *L. dahli* are widely distributed in central Armenia to southern Georgia and *L. mixta* is currently restricted to upper Kura River valley and the Black Sea coastal region of western Georgia.

Genetic investigations have found little allozyme variation among populations of *L. armeniaca* and *L. dahli*. MacCulloch et al. (1995) and Fu et al. (in press) reported four clones in *L. armeniaca*, with one widespread clone and three rare clones. Murphy et al. (1997) reported five clones in *L. dahli*, among which one was widespread, and the other four restricted to one or two individuals.

Comparisons of mtDNA variation in the maternal parental species and their parthenogenetic daughter species have advanced our understanding about the age and modes of origin of parthenogenesis. Moritz et al. (1992) first estimated the extent of mtDNA divergence using restriction enzymes. Fu (1999) analyzed cytochrome-*b* (cyt-*b*) variation in *L. raddei* and its parthenogenetic daughter species. This study examines the other maternal parental species, *L. mixta*, and its daughter species using cyt-*b* and ATPase 6 gene sequences.

Materials and methods

Two populations of *L. mixta*, six of *L. dahli*, and seven of *L. armeniaca* were examined. Three specimens from each population of *L. mixta* and one specimen each from populations of *L. dahli* and *L. armeniaca*

were sequenced. Voucher specimens and locality data are listed in Appendix I.

Standard phenol–chloroform methods were used to extract DNA from tail muscle or liver tissues. Laboratory protocols follow Palumbi (1996). Polymerase chain reaction (PCR) was used for amplifying the DNA samples; parameters and settings follow Palumbi (1996). PCR products were isolated by electrophoresis on a 1.5% agarose gel followed by purification using GeneClean (Bio101) procedure. P^{33} labeled terminator cycle sequencing kits (Amersham) were used for DNA sequencing. Protocols followed manufacturer's recommendations with minor modification. The cycling parameters were set to 95°C for 30 s, 55°C for 30 s, 72°C for 60 s, and 30 cycles. Appendix II presents the primers used for PCR and sequencing the target ATPase 6 and cyt-*b* gene segments. All sequences were completed for both directions with 80–90% overlap. DNA sequences were edited in ESEE (version 3; Cabot & Beckenbach, 1989).

Nucleotide statistics, including base composition and substitution patterns, were computed using MEGA (version 1.01; Kumar, Tamura & Nei, 1993). The divergence of populations and haplotypes was measured by direct pairwise comparison.

Results

A 1044 base pair (bp) fragment of cyt-*b* was amplified and sequenced, and a 596 bp fragment of ATPase 6 was amplified and sequenced for all specimens. All sequences are deposited in GenBank (accession numbers AF147796-AF147805). Among the six specimens of *L. mixta*, eight sites were variable in cyt-*b*, and three in ATPase 6. These variable sites sorted the six samples into three haplotypes (Appendix III). Haplotype A, B and C consisted of three, two and one specimens, respectively. Haplotype A and B occurred in both Achaldaba and Bakuriani populations, while C only occurred in Bakuriani population. Figure 1 illustrates the relationships among the three haplotypes. The largest pairwise difference among the six samples was 0.61%.

All variations occurred in third codon positions, and all were transitions. In cyt-*b*, T-C and A-G substitutions each consisted of half of the eight substitutions. All three substitutions were A-G in ATPase 6. No protein level variation was observed. No insertions or deletions were found.

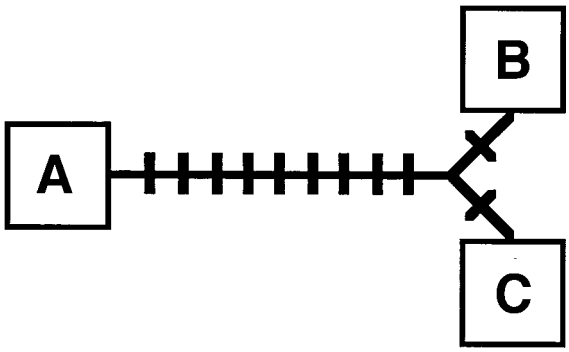

Figure 1. The relationships among the three haplotypes of *Lacerta mixta*, *L. armeniaca* and *L. dahli*. Each bar represents an assuming substitution. A = *L. mixta* of Achaldaba ($n = 2$) and Bakuriani ($n = 1$); all *L. armeniaca* and all *L. dahli*; B = *L. mixta* of Achaldaba ($n = 1$) and Bakuriani ($n = 1$); C = Bakuriani ($n = 1$).

No sequence variation was found among populations of parthenogenetic *L. armeniaca* and *L. dahli*. Surprisingly, all 13 populations of *L. armeniaca* and *L. dahli* were identical for both cyt-*b* and ATPase 6. Both species shared haplotype A of *L. mixta* for both genes (Appendix III).

The cyt-*b* nucleotide composition of these three species was similar to that in the *L. raddei* complex (Fu, 1999). In *L. mixta*, the third codon position was strongly biased against guanine (A:T:C:G = 33.4:27.5:36.7:2.4); the second codon position was thymine-rich (A:T:C:G = 20.4:42.0:25.3:12.4); and the first codon position were relatively equally distributed of the four bases (A:T:C:G = 27.7:23.6:28.7:20.0). The composition of the ATPase 6 gene in *L. mixta* was similar to cyt-*b*. The A:T:C:G of first, second and third codon positions were 37.2:18.7:28.3:15.8, 12.6:46.0:30.8:10.6, and 45.4:23.1:27.6:3.9, respectively. The nucleotide composition of the two parthenogens was largely the same as *L. mixta*.

Discussion

Divergence in *L. mixta* is extremely low compared to that of *L. raddei*. For example, the largest pairwise difference for cyt-*b* among *L. mixta* is 0.77%, while it is 7.76% in *L. raddei* (Fu, 1999). The seemingly limited divergence in *L. mixta* may partially result from the small numbers of populations included in this study ($n = 2$), and the short geographic distance between the two populations ($\approx$ 30 km). The distribution of *L. mixta* is limited to a small area of western Republic of Georgia. Unfortunately, political instability

in the Republic of Georgia limited the scope of the field collecting. Nevertheless, although more samples are desirable, a significant increase in observed divergence seems unlikely considering the relatively restricted distribution of *L. mixta*. Furthermore, *L. mixta* likely experienced several bottleneck effects resulting from multiple Pleistocene glacial events in Caucasus region.

Neither parthenogenetic *L. armeniaca* nor *L. dahli* showed any intraspecific variation (Appendix III). Our samples used in this study were collected across the ranges of the two parthenogens, and thus should accurately represent the divergence within the species. The lack of divergence among the two parthenogens is concordant with the results of Moritz et al. (1992). Using restriction enzymes, Moritz et al. (1992) found that all 17 samples of *L. dahli* and 19 of the 20 samples of *L. armeniaca* shared identical patterns. The single variant of *L. armeniaca* was estimated of having 0.2% sequence divergence. This scenario suggested that each parthenogenetic species was likely derived from one F_1hybrid or a few F_1 hybrids with the same or closely related maternal parents.

No interspecific mtDNA sequence variation was observed between *L. armeniaca* and *L. dahli*. However, the two parthenogens have different paternal parents, *L. valentini* and *L. portschinskii*, respectively. Therefore, they originated from at least two different hybridization events. The females of *L. mixta* involved in the two hybridization events had either the same or extremely similar mitochondrial genomes. This lack of interspecific variation in *L. armeniaca* and *L. dahli* may reflect, at least partially, the low level of variation in the maternal parent *L. mixta*. An alternative, however less likely, explanation is that the maternal parents of the two species were from the same population; this scenario implies that the two species originated from the same place at the same time. In comparison, great intraspecific variation occurs in *L. raddei* and among some of its daughter unisexual species. In particular, parthenogenetic species with different paternal parents displayed substantial mtDNA variation (e.g. 2.97% difference between *L. rostombekowi* and *L. sapphirina*; 3.35% between *L. rostombekowi* and *L unisexualis*; Fu, 1999).

Parthenogenetic *L. armeniaca* and *L. dahli* showed no divergence from the maternal parents implying a recent origin. In comparison, *L. raddei* and its daughter species showed greater variation (e.g., L. *rostombekowi* vs. *L. raddei* from Egagnadzor, 1.05%; *L. sapphirina* vs. *L. raddei* from Muradiye, 0.53%; Fu, 1999). Thus, the origins of *L. armeniaca* and *L. dahli* are likely to be much more recent than the formations of the other five parthenogenetic Caucasian rock lizards.

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Appendix I. Specimen examined

L. mixta ($n = 6$) – ROM24369, ROM24366, ROM24367, Georgia: Achaldaba, 41°54′24″N, 043°31′05″E; ROM26604, ROM26605, ROM26606, Georgia, Bakuriani, 41°40′N, 043°30′E.

L. dahli ($n = 6$) – ROM24078, Armenia: Papanino, 40°44′N, 044°49′E; ROM24031, Armenia: Stepanavan 41°01′15″N, 044°22′54″E; ROM24939, Armenia: Tumanyan, 41°00′00"N, 044°40′12″E; ROM26529, Georgia: Kodjovi, 41°38′32″N, 044°41′02″E; ROM26547, Georgia: Kareli, 42°01′N, 043°52′E; ROM26562, Georgia: Manglisi, 41°43′N, 044°25″E.

L. armeniaca ($n = 7$) – ROM24133, Armenia: Sevan, 40°30′59″N, 044°56′16″E; ROM24118, Armenia: Papanino, 40°44′N, 044°49′E; ROM24152, Armenia: Ankavan, 40°38′15″N, 044°32′54″E; ROM24192, Armenia: Steponavan, 41°01′15″N, 044°22′54″E; ROM24753, Armenia: Sevan Pass 40°41′12″N, 044°51′20″E; ROM24979, Armenia:

Cytochrome-*b*

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Type A    TTT GGG TCA TTA CTA GGA CTC TGC CTC ATC ATC CAA ACC ATC ACA GGC CTC TTC CTA GCT ATA CAT TAC ACT GCA GAC ATC ATA TCC GCA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    TTT TCA TCT ATT GCC CAC ATC CAC CGA GAC GTC CAA CAC GGA TGA TTA ATC CGT AAC CTG CAT GCT AAT GGC GCA TCC ATA TTC TTT ATC
Type B    ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    TGC ATT TAC CTT CAC ATC GGA CGT GGC CTA TAC TAT GGT TCC TAT ATT TAT ACT GAA ACC TGA AAC ATC GGA ATT ATT CTA CTC CTC CTA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    GTA ATA GCC ACA GCC TTT ATA GGC TAC GTC CTA CCC TGA GGA CAA ATA TCC TTT TGA GGG GCC ACC GTC ATT ACT AAT CTC CTC TCT GCA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    ATA CCC TAT GTA GGC TCA ACC CTT GTA GAG TGA ATT TGG GGT GGA TTT GCA ATC GAT AAC GCA ACC CTA ACT CGA TTC TTC ACC CTT CAC
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    TTT ATA CTT CCT TTT ATC ATT ATG GGC ACT TCA ATA ATT CAT CTA TTA TTT CTT CAT GAA ACA GGC TCT AAC AAC CCA GCA GGC CTT AAT
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    TCT AAT ACA GAT AAA ATC CCA TTC CAT CCC TAC TAC TCC TAC AAA GAC CTT TTA GGT GCC CTT ACC ATA CTA CTA ACC CTC CTC CTC CTA
Type B    ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    ACT CTC TTT TCA CCT AAC CTC CTA GGA GAC CCA GAA AAT TTC TCC CCT GCA AAC CCC CTG GTC ACC CCT CCC CAC ATT AAA CCA GAG TGA
Type B    G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    TAT TTC CTC TTC GCC TAT GCA ATC CTT CGT TCT ATT CCT AAT AAA CTA GGC GGT GTC CTA GCC CTC CTC TTC TCA ATC CTA GTC CTT CTA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ...

Type A    ATT ATA CCC CTA ACA CAC CTA TCA AAA CAA CGC ACT CTA TCC TAC CGC CCA CTA TCC CAA ACA CTT TTC TGA CTT TTA ATC TCA GAC ATT
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    ATC ATC TTA ACC TGA ATT GGA GGC CAA CCA GTA GAG CAC CCA TTT ATT ATT ATT GGT CAA CTA GCT TCT ACA TTT TAC TTT TTA ACT TTC
Type B    ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ...

Type A    CTT ATT TTT ATA CCC ACT ATT GCC CTA ATA GAA AAC AAA CTA CTC AAA TCA TAA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
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ATPase 6

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Type A    TGT ATC CCA AGC CTC CTA GGA GTA CCT TTA ATT ATA CTA GCT TTA TTT TTC CCA CTA ATA ATC TGA TTC ACA ACT AAC CGC CTC ATC CAA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    AAT CGA TAC TCA ACT ATT CAA TCC TCA CTT CTT ACT TAT ATT ACA AAA CAA ATA ATA TTA CCA ATT AAT ATT TCA GGC CAC AAA TGA GCA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ...

Type A    AGT ACC TTC ATC ACA CTA ATA CTA ATA CTC ATA CTA CTT AAC ACC CTG GGC CTT CTA CCA TAT ACT TTT ACC CCC ACC ACC CAA CTC TCA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    ATA AAT ATA GCT CTT GCC ATG CCA GCT TGA TTA ATA ACA GTT TTA ACT GGG CTA CGA AAT CAA CCC ACA ACC TCA TTA GGC CAC CTC CTA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ...

Type A    CCA GAG GGC ACA CCC ATC TTA TTA ATT CCT ATG TTA GTT TTA ATC GAA ACA GCT AGC TTA CTC ATC CGC CCA ATT GCT TTA GGC GTA CGA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    CTA ACA GCC AAC CTA ACA GCC GGA CAC CTA TTA ATT CAA CTT ACC TCA ACA GCA GTA CTT GCT CTA ATA AAT ACC ATA ACC ACT ACC GCA
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Type A    ATA ATT ACC CTA TTG ATA CTC ATT TTA TTA TCC TGT CTG GAA GTG GCC GTT GCC
Type B    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Type C    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
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Kutchak, 40°18′N, 043°40′E; ROM24997, Armenia: Tumanyan, 41°00′00″N, 044°40′12″E; ROM26514, Georgia: Bakuriani, 41°40′N, 043°30′E.

Appendix II. Primers used for amplifying and sequencing ATPase 6 and cyt-*b* segments

Letters L and H refer to light and heavy strands, and the numbers refer to the position of the 3′ ends of the primers in the complete human mtDNA sequence (Anderson et al., 1981).

H9148 5′ ACG AAT ACG TAG GCT TGG ATT A 3′, L15153 5′ TGA GGA CAA ATA TCC TTC TGA GG 3′, L15369 5′ CAT GAA ACT GGA TCA AAC AAC CC 3′, This study; L8552 5′ ATG AAC CTA AGC TTC TTC GAC CAA TT 3′, H8956 5′ ATA AAA AGG CTA ATT GTT TCG AT 3′, H15488 5′ TTG CTG GGG TGA AGT TTT CTG GGT C 3′, H15915 5′ GTC TTC AGT TTT TGG TTT ACA AGA C 3′, O. Haddrath (personal communication); L14841 5′ CCA TCC AAC ATC TCA GCA TGA TGA AA 3′, H15149 5′ GCC CCT CAG AAT GAT ATT TGT CCT CA 3′, Kocher et al. (1989).

Appendix III. The cyt-*b* and ATPase 6 gene sequences of *Lacerta mixta* and its parthenogenetic daughter species

Haplotype A = *L. mixta*: ROM24369, ROM24366, ROM26604; All *L. armeniaca*; All *L. dahli*; Haplotype B = *L. mixta*: ROM24367, ROM26606; Haplotype C = *L. mixta*: ROM26605.

References

Anderson, S., A.T. Bankier, B.G. Barrell, et al. (14 co-authors), 1981. Sequence and organization of the human mitochondrial genome. Nature 290: 457–465.

Cabot, E.L. & A.T. Beckenbach, 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Comp. Appli. Biol. 5: 233–234.

Darevsky, I.S., 1992. Evolution and ecology of parthenogenesis in reptiles, pp. 21–39 in Herpetology: Current Research on the Biology of Amphibians and Reptiles. Proceedings of the First World Congress of Herpetology, edited by K. Adler. Soc. Study Amphib. Rept., Oxford.

Darevsky, I.S., L.A. Kupriyanova & T. Uzzell, 1985. Parthenogenesis in reptiles, pp. 411–526 in Biology of the Reptilia, vol. 15, edited by C. Gans and F. Billett. Wiley, New York.

Fu, J., 1999. Phylogeny of lacertid lizards (Squamata: Lacertidae) and the evolution of unisexuality. PhD dissertation. University of Toronto.

Fu, J., R.D. MacCulloch, R.W. Murphy & I.S. Darevsky. Clonal variations in cancasian rock lizard *Lacerta armeniaca* and its origins. Amphibia-Reptilia (in press).

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Pääbo, F.X. Villablanca & A.C. Wilson, 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. 86: 6196–6200.

Kumar, S., K. Tamura & M. Nei, 1993. MEGA: Molecular Evolutionary Genetics Analysis, Version 1.01. The Pennsylvania States University, University Park, PA 16802.

MacCulloch R.D., R.W. Murphy, L.A. Kupriyanova, I.S. Darevsky & F.D. Danielyan, 1995. Clonal variability in the parthenogenetic rock lizard, *Lacerta armeniaca*. Genome 38: 1057–1060.

Moritz, C., W.M. Brown, L.D. Densmore, J.W. Wright, D. Vyas, S. Donnellan, M. Adams & P. Baverstock, 1989. Genetic diversity and the dynamics of hybrid parthenogenesis in *Cnemidophorus* (Teiidae) and *Heteronotia* (Gekkonidae), pp. 87–112 in Evolution and Ecology of Unisexual Vertebrates, edited by R.M. Dawley and J.P. Bogart. New York State Mus. Bull. 466, New York.

Moritz, C., T. Uzzell, S. Spolsky, H. Hotz, I. Darevsky, L. Kupriyanova & F. Danielyan, 1992. The material [sic maternal] ancestry and approximate age of parthenogenetic species of Caucasian rock lizards (*Lacerta*: Lacertidae). Genetica 87: 53–62.

Murphy, R.W., I.S. Darevsky, R.D. MacCulloch, J. Fu, L.A. Kupriyanova, D.E. Upton & F. Danielyan, 1997. Old age, multiple formations or genetic plasticity? Clonal diversity in a parthenogenetic Caucasian rock lizard, *Lacerta dahli*. Genetica 101: 125–130.

Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction, pp. 205–247 in Molecular Systematics, 2nd edition, edited by D.M. Hillis, C. Moritz and B.K. Mable. Sinauer, Sunderland, Massachusetts.

Schmidtler, J.F., J. Eiselt & I.S. Darevsky, 1994. Untersuchungen an Felseidechsen (*Lacerta-saxicola*-Gruppe) in der östlichen Türkei: 3. Zwei neue parthenogenetische Arten. Salamandra 30: 55–70.