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Divergence of the Cytochrome *b* Gene in the *Lacerta raddei* Complex and Its Parthenogenetic Daughter Species: Evidence for Recent Multiple Origins

JINZHONG FU, ROBERT W. MURPHY, AND ILYA S. DAREVSKY

Questions concerning the origin of parthenogenesis in Caucasian Rock Lizards and genetic divergence among bisexual lizards of the *Lacerta raddei* complex were examined using sequences from the mitochondrial cytochrome *b* gene. The maternal parent of the parthenogenetic *L. uzzelli, L. sapphirina,* and *L. bendimahiensis* was confirmed to be *L. raddei*. Although substantial variation was revealed among bisexual populations of *L. raddei* and *L. nairensis,* very low or no variation was found among the parthenogenetic species. A phylogenetic tree including 11 populations of *L. raddei* and *L. nairensis,* as well as 10 populations of its five daughter parthenogens, was constructed. Because of paraphyletic relationships, *L. nairensis* is considered conspecific with *L. raddei*. Evaluation of the parthenogenetic species suggests that separate hybridization events between *L. raddei* and *L. valentini* might have occurred at least twice. One resulted in *L. sapphirina* and *L. bendimahiensis,* and the other one (or more) resulted in *L. unisexualis* and *L. uzzelli*. The females involved were distantly related. *Lacerta unisexualis* and *L. uzzelli* likely had separate origins, but the females involved were closely related.

PARTHENOGENESIS in Caucasian Rock Lizards (genus *Lacerta*) has intrigued biologists since it was discovered nearly a half century ago (Darevsky, 1957, 1958). Parthenogenetic species originate from interspecific hybridization between bisexual species, and the eggs, produced without meiosis, develop into genetically identical offspring (Uzzell and Darevsky, 1975; Darevsky et al., 1985). The *Lacerta raddei* complex, including *L. r. raddei, L. r. vanensis,* and *L. nairensis,* has been identified as the maternal parent of five (of seven) parthenogenetic species: *L. rostombekowi, L. unisexualis, L. uzzelli, L. sapphirina,* and *L. bendimahiensis* (Darevsky and Danielyan, 1977; Darevsky, 1992; Schmidtler et al., 1994). Figure 1 depicts the hypothetical parentage of the seven parthenogenetic species of Caucasian rock lizards (Darevsky et al., 1985; Moritz et al., 1992; Schmidtler et al., 1994).

A recent series of molecular studies have examined population variation in Caucasian rock lizards. Moritz et al. (1992) using mtDNA restriction fragment analysis, and MacCulloch et al. (1995, 1997), Murphy et al. (1997), and Fu et al. (1998) employing allozyme electrophoresis, examined divergence of the parthenogenetic species. Little variation was found. For example, *L. rostombekowi* expressed no variation in or among the four populations studied (MacCulloch et al., 1997). Bobyn et al. (1996) found little intrapopulation genetic variation in *L. raddei* and *L. nairensis,* two proposed parental forms, although both species displayed relatively high levels of substructuring among populations. In addition, all but one of the originally proposed bisexual parents were confirmed; Moritz et al. (1992) proposed that *L. valentini* was the maternal parent of *L. uzzelli,* whereas Darevsky and Danielyan (1977) proposed that it was *L. nairensis.*

The species status of *L. nairensis* has been questioned recently. Bobyn et al. (1996) examined 36 allozyme loci but found no fixed allelic difference between *L. raddei* and *L. nairensis.* However, in this study, we treated *L. nairensis* as a valid species, and the monophyly of the two species was tested while examining the divergence of the *L. raddei* complex.

The main objectives of this study are to examine the diversity of cytochrome *b* (cyt *b*) gene sequences within the *L. raddei* complex and its parthenogenetic daughter species. Furthermore, phylogenetic relationships among the mtDNA haplotypes are established, and questions pertinent to the origin of parthenogenesis in Caucasian rock lizards are addressed.

MATERIALS AND METHODS

Five populations currently assigned to *L. raddei* and six currently assigned to *L. nairensis* were examined along with four populations of *L. unisexualis,* three of *L. rostombekowi,* and one each of *L. uzzelli, L. sapphirina,* and *L. bendimahiensis.* Moritz et al. (1992) found that the estimates of mtDNA nucleotide diversity (π) within popula-

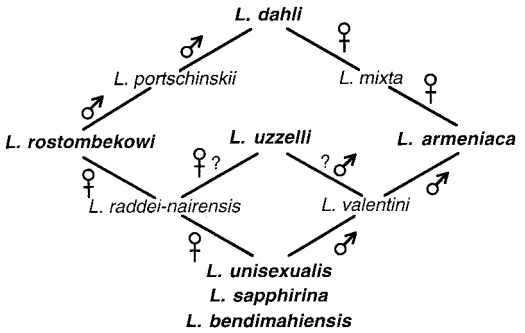

Fig. 1. Hypothetical parentage of parthenogenetic Caucasian rock lizards. Species in bold are parthenogens, and others are the bisexual parental species. "?" indicates uncertainty.

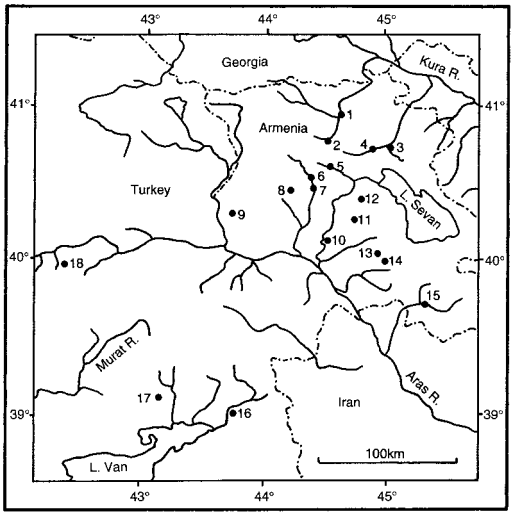

Fig. 2. Map of Transcaucasus with the distribution of localities from which sexual and parthenogenetic species were collected. The numbers at each locality correspond to those in the Appendix.

tions were significantly lower than among populations in Caucasian rock lizards (0.003 vs 0.018 in *L. nairensis* and 0.000 vs 0.031 in *L. raddei*). Bobyn et al. (1996) also suggested that populations of *L. raddei* and *L. nairensis* are disjunct, with limited gene flow among them. Therefore, only one specimen from each population was sequenced. Three other species of rock lizards, *L. valentini, L. derjugini,* and *L. parvula,* were used for outgroup comparison. These three species represent the three clades that branch off sequentially prior to *L. raddei* complex (Fu et al., 1997). Therefore, they are the best choice of outgroups (Maddison et al., 1984). *Lacerta valentini* was also examined as a potential alternative maternal parent of *L. uzzelli*. Voucher specimens and locality data are listed in the Appendix and shown in Figure 2.

Blood, heart, liver, and tail muscle tissues were collected during 1992–1996 and frozen in liquid nitrogen for use in multiple molecular investigations. All voucher specimens were deposited in the herpetological collections of the Royal Ontario Museum. Standard phenol-chloroform method was used to extract DNA from tail muscle or liver tissues. Laboratory protocols follow Palumbi (1996). Mitochondrial gene cyt *b* was chosen as the molecular marker, because that cyt *b* is one of most well-studied genes and has been used in a large number of systematic and population studies, which facilitates across-taxa comparisons. After the initial sequencing, a pseudogene was found, which presumably was a nuclear copy of mtDNA cyt *b* gene. Purification of mitochondria was accomplished using the method presented by Palumbi (1996). This method successfully limited the nuclear DNA signal to an undetectable level on the sequencing gels.

Standard polymerase chain reaction (PCR) was used for amplifying the DNA samples with annealing temperature of 50 C. ^{33}P-labeled terminator cycle sequencing kits (Amersham) were used for DNA sequencing. Protocols followed manufacturer's recommendations with annealing temperature of 55 C. Six primers were used for amplifying and sequencing the target cyt *b* gene segments (Table 1). All sequences were generated in both directions with 80–90% overlap. DNA sequences were edited in ESEE3 (Cabot and Beckenbach, 1989).

The phylogenetic analysis using maximum parsimony was conducted with PAUP (vers. 3.1.1, D. L. Swofford, 1993, unpubl.). Bootstrap proportions (Felsenstein, 1985) were calculated with 1000 replicates. All 11 populations of *L. raddei* and *L. nairensis,* as well as all 10 parthenogenetic populations, were included in the tree construction. The well-established mother-daughter relationships (except *L. uzzelli*) and strict maternal inheritance of mtDNA warranted the monophyly of the ingroup. The divergence of populations was measured by *p*-distance in MEGA (vers. 1.01, S. Kumar, K. Tamura, and M. Nei, 1993, unpubl.), which is defined as the proportion (*p*) of nucleotide sites at which the two sequences compared are different.

The phylogenetic tree was used to test hypotheses relating to the origin of parthenogenesis. A single origin means that a parthenogenetic species (clone) formed from a single F_1 hybrid. Multiple origins means that the parthenogenetic species (clones) formed from several

TABLE 1. PRIMERS USED FOR AMPLIFYING AND SEQUENCING CYT *b* SEGMENTS IN THIS STUDY.

Human position[a]	Gene	Sequence	References
L14841	cyt *b*	5′ CCA TCC AAC ATC TCA GCA TGA TGA AA 3′	Kocher et al., 1989
H15149	cyt *b*	5′ GCC CCT CAG AAT GAT ATT TGT CCT CA 3′	Kocher et al., 1989
L15153	cyt *b*	5′ TGA GGA CAA ATA TCC TTC TGA GG 3′	Fu et al. (in press)
H15488	cyt *b*	5′ TTG CTG GGG TGA AGT TTT CTG GGT C 3′	O. Haddrath (pers. comm.)
L15369	cyt *b*	5′ CAT GAA ACT GGA TCA AAC AAC CC 3′	Fu et al. (in press)
H15915	$tRNA^{Glu}$	5′ GTC TTC AGT TTT TGG TTT ACA AGA C 3′	O. Haddrath (pers. comm.)

[a] 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).

F_1 hybrids. F_1 hybrids may be from a single hybridization event or from several events between the same bisexual parental species. By testing the monophyly of the parthenogenetic species, it can be determined whether parthenogens evolved from a single origin or multiple origins. Phylogenetic affiliation between the parthenogenetic species and one local maternal population will indicate a constrained, localized origin. The association of parthenogens with several divergent maternal populations will support multiple origins. The extent of divergence between maternal populations and parthenogenetic species should shed light on the age of the parthenogens.

RESULTS

A total of 1044 base pairs (bps) sequences were resolved for all 24 specimens; these correspond to positions 14843–15886 of the human mtDNA sequence (Anderson et al., 1981). Sequence data have been deposited in GenBank (accession numbers AF164073–91; U88606–7; U88609; U88611; U88613). All sequences can be translated into amino-acid residues with vertebrate mtDNA genetic code. Therefore, we assumed that the mtDNA cyt *b* sequences are neither a nuclear copy nor a pseudogene. The fragment constitutes approximately 91% of the cyt *b* gene. No insertions and/or deletions were found.

Phylogenetic analyses.—A total of 184 potentially phylogenetically informative characters were resolved. A parsimony analysis treating all characters as nonadditive (= unordered) and equally weighted resulted in two equally most parsimonious trees (MPTs) with 336 steps, CI of 0.622, and RI of 0.779. The strict consensus tree is shown in Figure 3. All ingroup taxa grouped into five clades on both trees. All populations of *L. unisexualis* and *L. uzzelli* were grouped with three populations of *L. nairensis* (Apnaguch, Adis II, and Aragatz). All populations of *L. rostombekowi* were grouped with the Egegnadzor population of *L. raddei*. *Lacerta sapphirina* and *L. bendimahiensis* were grouped with the Muradiye population of *L. raddei*. Two populations of *L. nairensis* (Yerevan and Adis I) were grouped with two populations of *L. raddei* (Geghart and Chosrov). The Gosh population of *L. raddei* was grouped with Tumanyan population of *L. nairensis*. The relationships within each of the five major clades were identical between the two trees (Fig. 3). The differences were the relationships between the five clades. MPT-1 placed the Gosh-Tumanyan population clade at the base of

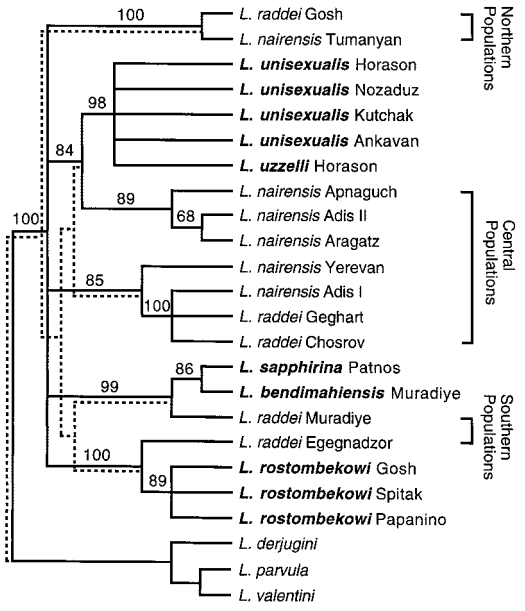

Fig. 3. The phylogenetic relationships of the populations of *Lacerta raddei* complex and its parthenogenetic daughter species. The solid lines represent the strict consensus tree of the two equally most parsimonious trees from the unweighted analysis, and the dashed lines represent the single most parsimonious tree from the weighted analysis. Species in bold are parthenogens. Numbers above the lines are bootstrap proportions greater than 0.50 on the consensus tree, which are derived from 1000 replicates.

the tree and grouped the two southern populations, Egegnadzor and Muradiye, together as sister groups. MPT-2 placed Egegnadzor and Muradiye populations next to each other at the base of the tree. Bootstrap proportions over 0.50 are mapped on the consensus tree (Fig. 3).

To further resolve the relationships among the five clades, a character weighting scheme was applied. Transversion changes are less likely to occur than transitions, and they are also less frequently observed in nature. Giving high weight to transversion change would more accurately reflect the genealogical relationships, especially in the cases of deep divergence (Hillis et al., 1994). The observed transversion:transition ratio on the two MPTs are 45:254 and 42:232, respectively. Therefore, we inversely weighted transversions by 6 according to the ratio. One MPT resulted, which was two steps longer than the first two MPTs when all characters were weighted by one (Fig. 3). The relationships within the five major clades are identical to the two previous MPTs. It is most similar to MPT-1 in terms of the basal position of the Gosh-Tumanyan population clade and the sister-group relationship of the Muradiye and Egegnadzor populations. Moreover, the Apnaguch population, etc., clade and Yerevan population, etc., clade grouped together.

The parthenogenetic species did not cluster together but rather formed three groups on the tree. *Lacerta unisexualis* and *L. uzzelli* grouped together, and they were most closely related to populations of *L. nairensis* from Apnaguch, Adis II, and Aragatz. *Lacerta sapphirina* and *L. bendimahiensis* grouped together, and they clustered most closely with *L. raddei* from Muradiye. The three populations of *L. rostombekowi* clustered most closely with *L. raddei* from Egegnadzor, which agrees with Moritz et al. (1992).

Populations assigned to *L. raddei* did not cluster together. Neither did the populations assigned to *L. nairensis*. The population of *L. raddei* from Gosh was most closely associated with the population of *L. nairensis* from Tumanyan, rather than other populations of *L. raddei*. Populations of *L. nairensis* from Yerevan and Adis I were most closely clustered with populations of *L. raddei* from Chosrov and Geghart, not other populations of *L. nairensis*.

Variability within species groups and between parthenogens and their closest maternal populations.—Among the populations of *L. raddei* and *L. nairensis*, the greatest *p*-distance (0.0747–0.776) occurred between the northern populations (Gosh and Tumanyan) and southern populations (Muradiye and Egegnadzor). The smallest *p*-distance occurred between populations from Chosrov and Geghart (0.0019). The average distance among those populations was 0.0384 (Table 2).

The *p*-distances within and between parthenogenetic species are summarized in Table 2, along with average *p*-distances between the parthenogens and their closest "sister taxa." No variation was detected among the four populations of *L. unisexualis* or the three populations of *L. rostombekowi*. *Lacerta uzzelli* was identical to *L. unisexualis* but differed from *L. valentini* by 14.4%. *Lacerta sapphirina* was most similar to *L. bendimahiensis* with one bp difference (T–C, third codon position). The distances between *L. sapphirina–L. bendimahiensis* clade and *L. unisexualis–L. uzzelli* clade were between 0.0364 and 0.0374, which were close to the average distance among populations of *L. raddeia* and *L. nairensis*. The distances between parthenogens and their closest maternal populations were between 0.0048 and 0.0125, which were much less than the average distance among populations of *L. raddei* and *L. nairensis*.

At the protein level, a total of 24 sites (of 348)

TABLE 2. THE MEAN WITHIN-SPECIES *p*-DISTANCE AMONG POPULATIONS OF *Lacerta raddei* AND *L. nairensis* AND THE PARTHENOGENS, AND THE AVERAGE *p*-DISTANCE BETWEEN THE PARTHENOGENS AND THEIR CLOSEST MATERNAL POPULATIONS, AND BETWEEN THE PARTHENOGEN CLADE MEMBERS.

	Mean (±SD)	Range
L. raddei-nairensis	0.0384 ± 0.0204	0.0019–0.0776
L. unisexualis-L. uzzelli	0.0000 ± 0.0000	—
L. sapphirina-L. bendimahiensis	0.0010	—
L. rostombekowi	0.0000 ± 0.0000	—
L. unisexualis-L. uzzelli vs *L. nairensis* Apnaguch, Adis II, Aragatz	0.0109 ± 0.0015	0.0096–0.0125
L. sapphirina-L. bendimahiensis vs *L. raddei* Muradiye	0.0053 ± 0.0006	0.0048–0.0057
L. rostombekowi vs *L. raddei* Egegnadzor	0.0105 ± 0.0000	0.0105–0.0105
L. sapphirina-L. bendimahiensis vs *L. unisexualis-L. uzzelli*	0.0369 ± 0.0005	0.0364–0.0374

was variable among *L. raddei* and *L. nairensis* populations, and no variation was found among parthenogenetic species. Between the parthenogens and their closest maternal forms, there was one amino-acid residue difference between populations of *L. raddei* from Adis II, Apnaguch, and Aragatz, and the parthenogenetic *L. unisexualis* and *L. uzzelli*. It was derived from a second codon position substitution. Two amino-acid differences were detected between the pair of parthenogenetic *L. sapphirina* and *L. bendimahiensis* and the bisexual *L. raddei* from Muradiye. No differences separated *L. rostombekowi* and *L. raddei* from Egegnadzor.

DISCUSSION

Divergence of L. raddei complex.—Compared to other taxa of lower vertebrates, the *L. raddei* complex showed slightly higher levels of divergence (p-distance$_{max}$ = 0.0776). For example, the Sand Darter Fish, *Etheostoma vitreum*, had three variable sites in 402 bp (p-distance = 0.0075) among its populations (Wiley and Hagen, 1997); the Rainbow Fish, *Melanotaenia splendida splendida*, had less than 4% divergence (p-distance < 0.04) among populations (Zhu et al., 1994). Moritz et al. (1989) reported that the greatest intraspecific mtDNA divergence among *Cnemidophorus* was 6.7% (p-distance = 0.067). However, lizards from populations in close geographic proximity often had less than 1% divergence of mtDNA, which is typical for terrestrial vertebrates.

Populations assigned to *L. raddei* and *L. nairensis* did not form respective monophyletic groups. This finding is concordant with the allozyme study of Bobyn et al. (1996), which suggested that *L. nairensis* is conspecific with *L. raddei*.

Parentage of the parthenogens.—*Lacerta raddei* is the maternal parent of *L. unisexualis* and *L. rostombekowi*, with *L. valentini* and *L. portschinskii* being the paternal parents, respectively (Darevsky, 1992; MacCulloch et al., 1997; Fu et al., 1998). This study identified *L. raddei* as the maternal parent of *L. uzzelli*, and a recent allozyme study showed that *L. valentini* was the paternal species (J. Fu, R. D. MacCulloch, R. W. Murphy, I. S. Darevsky, and B. S. Tuniyev, unpubl.), as Darevsky and Danielyan (1977) originally proposed. In contrast, Moritz et al. (1992) concluded that *L. valentini* was the maternal parent because *L. uzzelli* and *L. valentini* showed an identical restriction fragment map. However, the specimens used by Moritz et al. (1992) and this study are from different populations. Assuming both findings to be correct, this discrepancy indicates interesting multiple origins for *L. uzzelli*, one in which *L. valentini* was the maternal parent and *L. raddei* the paternal species, and the other vice versa. Sampling more *L. uzzelli* populations is needed. The cyt *b* data also confirmed that *L. raddei* is the maternal parent of *L. sapphirina* and *L. bendimahiensis*, as predicted by Schmidtler et al. (1994). A recent allozyme study confirmed that *L. valentini* is also the paternal parent of *L. sapphirina* and *L. bendimahiensis* (J. Fu, R. D. MacCulloch, R. W. Murphy, I. S. Darevsky, and B. S. Tuniyev, unpubl.).

Phylogenetic tree.—Although the use of phylogenetic methods at the population level has been questioned (Nixon and Wheeler, 1990), they are suitable for this study. Although exceptions

do occur, mitochondrial DNA inheritance is predominantly maternal in animals, and no recombination has been detected (Zouros et al., 1994). Therefore, the resulting tree represents the genealogical relationships of the female haplotypes, and no reticulate evolution is involved. Moreover, the sampled bisexual populations are well isolated from one another, that is, there is an absence of, or very limited, gene flow. This is evidenced by the low intra- and high interpopulation variation, indicated by both mtDNA and allozyme data (Moritz et al., 1992; Bobyn et al., 1996). Consequently, males and females within a population likely experienced identical evolutionary pathways. Thus, the evaluation of cyt *b* presumably represents the genealogical history of the populations.

The phylogenetic tree grouped the Gosh and Tumanyan populations of *L. nairensis* together and placed this clade at the base of the weighted tree and one of the unweighted MPTs. These two populations occur on the northern slope of the Transcaucasus and are the only populations located in the Kura River drainage basin. Caucasian rock lizards typically require a habitat with high humidity (Darevskii, 1967). This trait has often constrained their distribution patterns to river valley systems. The absence of a direct valley connection appears to effectively isolate the Gosh and Tumanyan populations from the other populations.

The two southern-most populations, Egegnadzor and Muradiye, also grouped together on the weighted tree and one of the unweighted MPTs. Muradiye is in the Lake Van drainage basin, whereas Egegnadzor is in the Aras River basin. Geographically, Egegnadzor is closer to the central populations than it is to the Muradiye population. The association of the populations from Egegnadzor and Muradiye, if truly representing the genealogy, may indicate a relatively old historical connection as revealed by a medium level distance (*p*-distance = 0.0335). All the remaining seven populations are in central Armenia, in the Aras River system. They formed one group on the weighted tree, which was constituted by two major clades: populations from Apnaguch, Adis II, and Aragatz Mt. grouped together, as did populations from Geghart, Chosrov, Adis I, and Yerevan.

Origin of parthenogenesis.—Successful hybridization events between the same parental species, which led to the formation of multiple clones (or species) of parthenogens, have occurred more than once. The cyt *b* tree divided the parthenogens into three groups (Fig. 3): a *L. rostombekowi* clade, a *L. unisexualis–L. uzzelli* clade, and a *L. sapphirina–L. bendimahiensis* clade. The latter two clades share the same parental species, *L. raddei* and *L. valentini*, and they are closely associated with distantly related maternal populations. If the parents of *L. unisexualis* and *L. uzzelli*, as well as *L. sapphirina* and *L. bendimahiensis*, have been correctly identified, then successful hybridization between *L. raddei* and *L. valentini* must have happened at least twice. Furthermore, the distant relationship of the maternal ancestors shows that different populations were involved in the hybridization events, that is, the origins of the two parthenogenetic groups occurred in different regions and/or during different time periods. It is more likely that the hybridization events occurred at different locations, because of the close association of the parthenogens with different local populations (Fig. 3). Considering the genetic divergence between the parthenogens and their closest maternal populations, the hybridization event(s) leading to *L. unisexualis* and *L. uzzelli* probably happened much earlier than the one(s) leading to *L. sapphirina* and *L. bendimahiensis*. This is the first documented case in which different parthenogenetic species of vertebrates arose from multiple hybridization events involving distantly related females (and males), which has been reported in some gynogenetic and hybridogenetic vertebrates (Moritz et al., 1989). Multiple clones resulting from multiple origins have also been observed in parthenogenetic *Cnemidophorus tesselatus* and *Heteronotia binoei*, in which only closely related females were involved (Moritz et al., 1989). As a result, the pairwise mtDNA sequence differences among species (clones) of different origins varies substantially between the groups. They are 3.64% to 3.47% between the members of *Lacerta unisexualis–uzzelli* clade and *L. sapphirina–bendimahiensis* clade. In contrast, they are only 0.0% to 0.43% in *C. tesselatus* and 0.09% to 0.26% in *H. binoei* (Moritz et al., 1989).

A different population of *L. raddei* was involved in the hybridization event with *L. portschinskii*, which led to the formation of *L. rostombekowi* (Fig. 3). Extremely low variation in both mtDNA and nuclear genes (MacCulloch et al., 1997) suggested that *L. rostombekowi* likely originated from a single F_1 hybrid, that is, single origin.

Although *L. unisexualis* and *L. uzzelli* group together, indicating that their mtDNA share a common ancestor, it is unlikely that they share a single origin. *Lacerta unisexualis* and *L. uzzelli* are identical in terms of their mitochondrial DNA sequences obtained in this study. However, in *L. unisexualis*, the two nasal scales are in con-

tact, whereas they are separated by the rostral and frontonasal scales in *L. uzzelli* (Darevsky and Danielyan, 1977). Both scale patterns occur in each of their parental species. The absence of variation in scale pattern within the parthenogens suggests genetic control rather than epigenetic expression. Consequently, these two species likely had unique origins, despite our inability to distinguish between them genetically. One explanation is that the ancestors of the two species were F_1 siblings from same parents. The two siblings had identical mtDNA but different nuclear DNA resulting from heterozygous nuclear DNA of their parents. Alternatively, the ancestors of the two species arose from two hybridization events. In this case, the females involved in the hybridization must be closely related, probably from the same population. The hybridization events also probably occurred within a short time period, which resulted in the identical cyt *b* sequences.

Not all currently recognized parthenogenetic species appear to have a unique origin. *Lacerta sapphirina* and *L. bendimahiensis* also grouped together on the tree (Fig. 3), suggesting that their mtDNA shared a common ancestor as well, although there is one bp difference between them. The species are distinguishable only by coloration (Schmidtler et al., 1994). However, recent field observations have revealed that coloration varies considerably. Preliminary allozyme examination of their nuclear genes also failed to distinguish between the two parthenogens (J. Fu, R. D. MacCulloch, R. W. Murphy, I. S. Darevsky, and B. S. Tuniyev, unpubl.). Given the lack of diagnostic anatomical characteristics, and near genetic identity, the origin of these two species is best explained by a single hybridizaton event. If so, the one-bp difference between the two parthenogens was likely the result of a point mutation. However, the alternative explanation of separate origins cannot be completely ruled out. Sampling more populations and sequencing more individuals of these two parthenogens would provide more evidence.

Based on the study of four parthenogens, *L. unisexualis*, *L. rostombekowi*, *L. dahli*, and *L. armeniaca*, R. W. Murphy, J. Fu, R. D. MacCulloch, I. S. Darevsky, and L. A. Kupriuanova (unpubl.) hypothesized that a single origin may be the rule for each species of parthenogenetic Caucasian rock lizard. Allozyme studies revealed that parthenogens with multiple clones were composed of one major clone with additional geographically restricted clones, the latter often involving only a few individuals. Rather than representing unique origins, it is more likely that the restricted clones were formed as mutations after the origin of the species (Murphy et al., 1997; Fu et al., 1998; R. W. Murphy, J. Fu, R. D. MacCulloch, I. S. Darevsky, and L. A. Kupriuanova, unpubl.). The cyt *b* data supported a single origin for each of *L. unisexualis* and *L. rostombekowi*. However, the data also revealed that multiple origins, involving both closely and distantly related females, also occurred in Caucasian rock lizards, resulting in several parthenogenetic species from the same parental species.

Low intraspecific variation and a high degree of similarity to their parental forms has been a general rule in parthenogenetic lizards (Moritz et al., 1989). These characteristics have been explained by young age (Darevsky et al., 1985, Moritz et al., 1989), genetic constraints (Moritz et al., 1989), ecological constraints (Vrijenhoek, 1989), and phylogenetic constraints (Fu, 1999; R. W. Murphy, J. Fu, R. D. MacCulloch, I. S. Darevsky, and L. A. Kupreyanova, unpubl.). Our cyt *b* data confirm this rule. Although the *L. sapphirina–L. bendimahiensis* clade only has one variable site, the *L. unisexualis–L. uzzelli* group and *L. rostombekowi* do not vary at all. This contrasts strongly with the substantial variation observed among populations of *L. raddei*. The average difference of only 1% between the parthenogens and their closest maternal population is much lower than the average divergence observed among populations of *L. raddei* (3.8%). This low level of divergence is also concordant with the allozyme data, which reveals very little clonal variation. Only one clone has been detected in *L. rostombekowi* (MacCulloch et al., 1997); three in *L. armeniaca* (MacCulloch et al., 1995), three in *L. unisexualis* (Fu et al., 1998); and five in *L. dahli* (Murphy et al., 1997).

Although all parthenogens are of recent origin, they may have largely different ages, and their ages may be much older than we currently perceived. Differences in *p*-distances between the parthenogens and their closest maternal populations suggest variation in the ages of the parthenogens. For example, *L. sapphirina* and *L. bendimahiensis* are probably much younger than *L. unisexualis*, *L. uzzelli*, and *L. rostombekowi*. Another two parthenogenetic species, *L. dahli* and *L. armeniaca*, showed identical cyt *b* and ATPase 6 sequences with their maternal species, *L. mixta* (Fu et al., in press), which suggests that their ages may be even younger. Darevsky et al. (1985) estimated that the parthenogenetic Caucasian rock lizards originated after the Pleistocene glaciation of the Caucasian mountains, 5000 to 7000 years ago. Irwin et al. (1991) provided an estimate of a divergence rate of ap-

proximately 10% per million years for the silent substitution at the third-codon position, based on mammalian cyt *b* data. If the rate is equally applicable to lizards, the ages of some parthenogenetic *Lacerta* should be much older than Darevsky et al. (1985) estimated. For example, *L. rostombekowi* would be approximately 200,000 years old (2% divergence).

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(JF, RWM) CENTRE FOR BIODIVERSITY AND CONSERVATION BIOLOGY, ROYAL ONTARIO MUSEUM, 100 QUEEN'S PARK, TORONTO, ONTARIO, CANADA M5S 2C6; AND (ISD) ZOOLOGICAL INSTITUTE, RUSSIAN ACADEMY OF SCIENCES, ST. PETERSBURG 119034, RUSSIA. PRESENT ADDRESS: (JF) MUSEUM OF VERTEBRATE ZOOLOGY, 3101 VALLEY LIFE SCIENCES BUILDING, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA 94720-3160. E-mail: (JF) jinzhong@uclink4.berkeley.edu. Send reprint requests to RWM. Submitted: 25 Jan. 1999. Accepted: 18 Aug. 1999. Section editor: J. D. McEachran.

APPENDIX

Specimens examined in this study. The numbers in the parentheses correspond to those in Figure 2.

Lacerta raddei (n = 5).—ROM23619, Armenia: Egegnadzor, 39°45′N, 045°08′E (15); ROM23681, Armenia: Chosrov National Park, 40°00′54″N, 044°54′56″E (14); ROM23629, Armenia: Geghart, 40°08′15″N, 044°49′06″E (13); ROM23736, Armenia: Gosh, 40°44′51″N, 045°01′26″E (3); ROM28241, Turkey: Muradiye, 39°00′N, 043°44′E (16).

Lacerta nairensis (n = 6).—ROM23805, Armenia: Adis Mt. II, 40°23′N, 044°42′E (12); ROM24780, Armenia: Tumanyan, 41°00′00″N, 044°40′12″E (1); ROM24843, Armenia: Apnaguch, 40°27′N, 044°22′E (7); ROM23801, Armenia: Adis Mt. I, southern slope of Gehaim ridge (11); ROM23819, Armenia: Aragatz Mt., 40°21′54″N, 044°15′12″E (8); ROM26609, Armenia: Yerevan, 40°11′50″N, 044°29′48″E (10).

Lacerta unisexualis (n = 4).—ROM24242, Armenia: Ankavan, 40°38′15″N, 044°32′54″E (5); ROM24985, Armenia: Kutchak, 40°18′N, 043°40′E (9); ROM26800, Armenia: Nozaduz, 40°30′N, 044°20′E (6); ROM28318, Turkey: Horason, 39°50′N, 042°20′E (18).

Lacerta rostombekowi (n = 3).—ROM23985, Armenia: Papanino, 40°44′N, 044°49′E (4); ROM24983, Armenia: Spitak, 40°51′N, 044°19′E (2); ROM Field 12134 (no voucher specimens available), Armenia: Gosh, 40°44′51″N, 045°01′26″E (3).

Lacerta uzzelli (n = 1).—ROM 28293, Turkey: Horason, 39°50′N, 042°20′E (18).

Lacerta bendimahiensis (n = 1).—ROM28249, Turkey: Muradiye, 39°00′N, 043°44′E (16).

Lacerta sapphirina (n = 1).—ROM28276, Turkey: Patnos, 39°14′N, 042°52′E (17).

Lacerta parvula (n = 1).—ROM 24384, Georgia: Achaldaba, 41°54′24″N, 043°30′05″E.

Lacerta derjugini (n = 1).—ROM 26585, Georgia: Bakuriani, 41°40′N, 043°30′E.

Lacerta valentini (n = 1).—ROM 23861, Armenia: Sevan, 40°30′59″N, 044°56′16″E.