Genetica **87**: 53-62, 1992.
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The material ancestry and approximate age of parthenogenetic species of Caucasian rock lizards (*Lacerta*: Lacertidae)

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Received 21 January 1992 Accepted in revised form 21 April 1992

Key words: Evolution, lizards, mitochondrial DNA, parthenogenesis, hybridization

Abstract

Restriction enzymes were used to assay variation among mitochondrial DNAs from parthenogenetic and sexual species of *Lacerta.* This permitted identification of the sexual species that acted as the maternal parent of the various hybrid-parthenogenetic lineages. *Lacerta mixta* was the maternal parent for both *L. dahli* and *L. armeniaca*, *L. valentini* was the maternal parent for *L. uzzelli*, and *L. raddei* was the maternal parent of *L. rostombekovi*. The maternal ancestry of *L. unisexualis* is not as clear. The sample of *L. nairensis* was very similar to one from a population of *L. raddei* and either species could be the maternal parent of *L. unisexualis.* The parthenogenetic species all had very low nucleotide diversity in absolute terms and in comparison to their sexual relatives. The close similarity between mtDNAs from the parthenogenetic species and their respective sexual maternal ancestor species provides strong evidence for the recent origin of the parthenogens. The low diversity of the parthenogens indicates that few females were involved in their origins; the maternal parents of *L. dahli* and *L. armeniaca* could have come from a single population. The patterns of mtDNA variation in *Lacerta* are very similar to those in *Cnemidophorus* and *Heteronotia*, establishing recent and geographically restricted origins as a general feature of parthenogenetic lizards.

Introduction

Studies of proteins or chromosomes have shown that virtually all of the 50 or so clonally reproducing species of vertebrates arose through hybridization (reviewed by Darevsky, Kupriyanova & Uzzell, 1985; Vrijenhoek *et al.*, 1989) and, accordingly, have high levels of heterozygosity. Genotypic diversity ranges from near nil in *Cnemidophorus neomexicanus* (Parker & Selander, 1984; Cole, Dessauer & Barrowclough, 1989) to a high level, mostly due to multiple origins, among geckos of the *Heteronotia binoei* complex (Moritz *et al.*, 1989b).

Extensive surveys of mitochondrial DNA (mtDNA) variation in *Cnemidophorus* and *Heteronotia* have consistently revealed very low levels of mtDNA variation among parthenogenetic populations (Vyas *et al.*, 1990; Moritz, 1991; reviewed by Moritz *et al.*, 1989a). The variation in parthenogenetic species is typically similar to that within populations of their sexual relatives and an order of magnitude less than that among sexual populations. In *Cnemidophorus tesselatus* (Densmore, Wright & Brown, 1989) and *Cnemidophorus uniparens* (Densmore *et al.*, 1989), mtDNA of the parthenogenetic species is virtually indistinguishable from that of the sexual species that acted as the maternal parent. The combined mtDNA evidence suggests that each of the parthenogenetic taxa, even those as

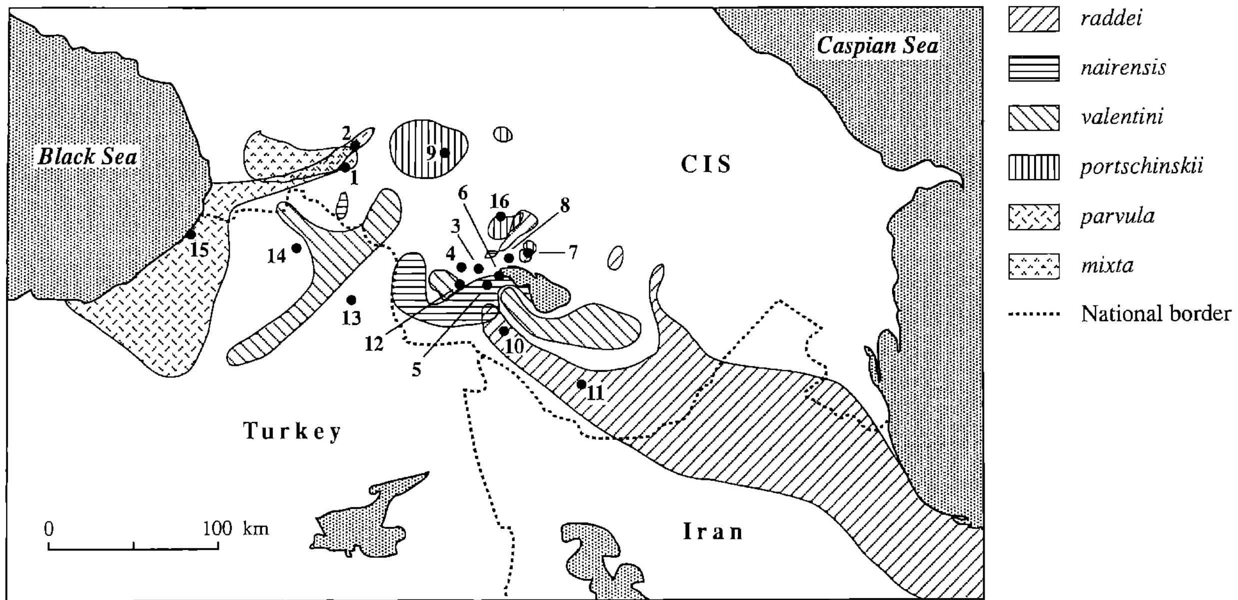

Fig. 1. Map of Caucasia showing ranges of sexual species involved in the origin of parthenogenetic species and the distribution of localities from which sexual and parthenogenetic *Lacerta* were sampled. Numbers at each locality correspond to those in Table 1. CIS corresponds to the Commonwealth of Independent States (formerly the USSR).

geographically widespread and genetically diverse as *Heteronotia binoei,* arose recently relative to the divergence of related sexual species. Furthermore, they probably each arose from a small geographic area (Moritz *et al.*, 1989a).

In this paper, we extend the study of mtDNA variation to parthenogenetic lineages of Caucasian rock lizards (genus *Lacerta*) and their sexual relatives. This genus is of special significance because these lizards have been the subject of long term ecological and biogeographic studies (Darevsky, 1966, 1967; Darevsky, Kupriyanova & Uzzell, 1985) and because parthenogenesis has arisen several times within the group (Darevsky, 1966, 1967; Uzzell & Darevsky, 1975). Data on distribution and electrophoretic variation in proteins demonstrated that the *Lacerta saxicola* group contains numerous sexual species and that four of the parthenogenetic species arose independently as a result of hybridization between different pairs of sexual species (Uzzell & Darevsky, 1975). Specifically, these data identified the sexual parents of *L. armeniaca* as *L. mixta* and *L. valentini*, of *L. dahli* as *L. mixta* and *L. portschinskii*, of *L. rostombekovi* as *L. raddei* and *L. portschinskii*, and of *L. unisexualis* as *L. raddei* and *L. valentini*. The subspecies of *L. raddei* involved in the ancestry of *L. unisexualis* and *L. rostombekovi* were tentatively identified as *L. r. nairensis* and *L. r. raddei*, respectively. Subsequently, Darevsky & Danielyan (1977) discovered a fifth parthenogenetic species, *L. uzzelli*, and suggested *L. parvula* and *L. r. nairensis* as possible parents.

The aims of the present study were (i) to identify the sexual species that acted as the maternal parent for each of the recognized parthenogenetic forms, and (ii) to estimate the diversity of mtDNA in the parthenogenetic species and their sexual relatives. From this information, we can make inferences about their phylogenetic and temporal origins and test the generality of the patterns observed in *Heteronotia* and *Cnemidophorus*.

Materials and methods

The 11 species examined are the five described parthenogenetic species of *Lacerta* (*L. armeniaca, L. dahli, L. rostombekovi, L. unisexualis, L. uzzelli*) and the six sexual species (*L. mixta, L. nairensis, L. parvula, L. portschinskii, L. raddei, L. valentini*) known or thought to have given rise to them. *L.*

Table 1. Specimens examined and estimates of mtDNA genome size. Sample sizes refer to individuals assayed with 4-bp or 6-bp recognizing enzymes, respectively. The numbers in parentheses after the localities correspond to points on the map in Fig. 1.

Species	Locality	mtDNA size (kb)	Sample 4bp	size 6bp
Parthenogens:				
L. unisexualis	Kars, Turkey (14)	21.5		1
	Ankavan, Armenia (4)	21.5	9	1
	Takyarlu, Armenia (3)	21.5	1	
	Hrazdan, Armenia (5)	21.5	4	
L. dahli	Dilijan, Armenia (8)	18.2	6	2
	Manglisi, Geogia (9)	18.2	11	
L. armeniaca	Takyarlu, Armenia (3)	18.2	2	
	Ankavan, Armenia (4)	18.2	3	1
	Hrazdan Armenia (5)	18.2	15	
L. rostombekovi	Dilijan, Armenia (8)	18.5	1	1
L. uzzelli	Ardahan, Turkey (7)	18.4	3	1
Bisexuals:				
L. raddei	Gehard, Armenia (10)	21.5	4	2
	Gosh, Armenia (7)	21.7	1	1
	Yegehnadzor, Armenia (11)	19.0	3	1
L. nairensis	Hrazdan, Armenia (5)	21.5	1	
	Sevan, Armenia (6)	21.5	3	1
L. mixta	Achaldaba, Georgia (2)	18.2	2	1
	Bakuriani, Georgia (1)	18.2	6	
L. valentini	Aragatz, Armenia (12)	18.4		1
L. parvula	Hopa, Turkey (15)	17.3		1
L. portschinksii	Stepanavan, Armenia (16)	17.6		1

nairensis is here treated as a species separate from *L. raddei* (J. Eiselt, I. S. Darevsky, and J. Schmidtler, in prep.). Eleven geographical samples of parthenogenetic lizards and 10 of sexual lizards were sampled from 16 localities in northwestern Turkey and the republics of Armenia and Georgia (Table 1; Fig. 1).

mtDNA of 79 specimens (one to 20 per species; Table 1) was prepared following either of two alternative methods. For specimens collected in 1979 and 1984, a 0.9/1.5M sucrose step gradient was used to obtain a fraction enriched in mitochondria. Mitochrondria in these crude preparations were lysed with SDS and the nuclear DNA precipitated with KAc to produce a crude lysate enriched for mtDNA (Spolsky & Uzzell, 1984). For more recently collected specimens, the step gradient was omitted and the basic protocol of Dowling, Moritz and Palmer (1990) was followed. Crude lysates produced by either method were centrifuged to equilibrium in CsCl gradients to separate supercoiled mtDNA from contaminating nuclear DNA (Dowling, Moritz & Palmer, 1990). Storage of crude lysates at −80 C for up to 10 years had no discernable effect on the yield or quality of mtDNA.

Samples of mtDNA were digested with restriction enzymes following suppliers' instruction. Fragments were end-labelled with ^{32}P-dNTPs, separated electrophoretically in 1.0% agarose and 3.5% polyacrylamide gels, and visualized by autoradiography (Brown, 1980; Dowling, Moritz & Palmer, 1990).

Most samples were cleaved with the 6-base enzyme, *Bcl*I. The sizes of fragments produced by *Bcl*I were estimated and summed to provide a measure of total mtDNA size. Subsequentlty, mtDNA from one or two individuals of each parthenogenetic species and of each geographically separated population of the seven sexual species was cleaved with 13 6-base enzymes (*Bam*HI, *Bcl*I, *Bgl*II, *Eco*RI, *Eco*RV, *Hind*III, *Nco*I, *Nhe*I, *Pvu*II, *Sac*II, *Sal*I, *Spe*I, and *Xba*I). For the sexual species *L. mixta, L. nairensis*, and *L. raddei* (three populations) and the parthenogenetic species *L. armeniaca, L. dahli, L. rostombekovi* and *L. unisexualis*,

the restriction sites for these 13 enzymes were mapped using double digests (Dowling, Moritz & Palmer, 1990). Comparisons with the sexual species *L. parvula, L. portschinskii* and *L. valentini* and the parthenogenetic species *L. uzzelli* were based on fragment patterns alone, rather than mapped sites. To assess variation of mtDNA within parthenogenetic and sexual species, 75 of the samples were also cleaved with six 4-base enzymes (*Dde*I, *Hin*fI, *Hin*PI, *Mbo*I, *Msp*I, and *Taq*I).

For mtDNAs that were mapped (Fig. 2), estimates of sequence divergence (δ) are based on the proportion of shared sites (Nei & Tajima, 1983). Comparisons among digests of very similar mtDNAs (i.e., $< 2\%$ sequence divergence) with 4-bp recognizing enzymes revealed few fragment differences, allowing individual site changes, and thus the proportion of shared sites to be deduced. Other comparisons using 4-bp and 6-bp recognizing enzymes used the proportion of shared fragments (F) to estimate sequence divergence (Upholt, 1977; Nei & Li, 1979) having identified the effects of length variation. Length variation was located by alignment of conserved sites across restriction maps. Fragments that differed only because they included variable-length regions and sites that mapped differently only because they surround variable-length regions were considered identical. Where less than 25% of fragments are shared ($F < 0.25$), the estimates of sequence divergence are subject to large errors (Kessler & Avise, 1985). Nucleotide diversity (π), the average number of differences per nucleotide site between mtDNAs randomly chosen from the populations, was calculated from divergence values as described by Nei and Li (1979). Phylogenetic analysis used the Exhaustive Search Algorithm of PAUP version 3.0 (Swofford, 1990), with Dollo parsimony and 100 bootstrap pseudoreplicates (Debry & Slade, 1985; Swofford & Olsen, 1990).

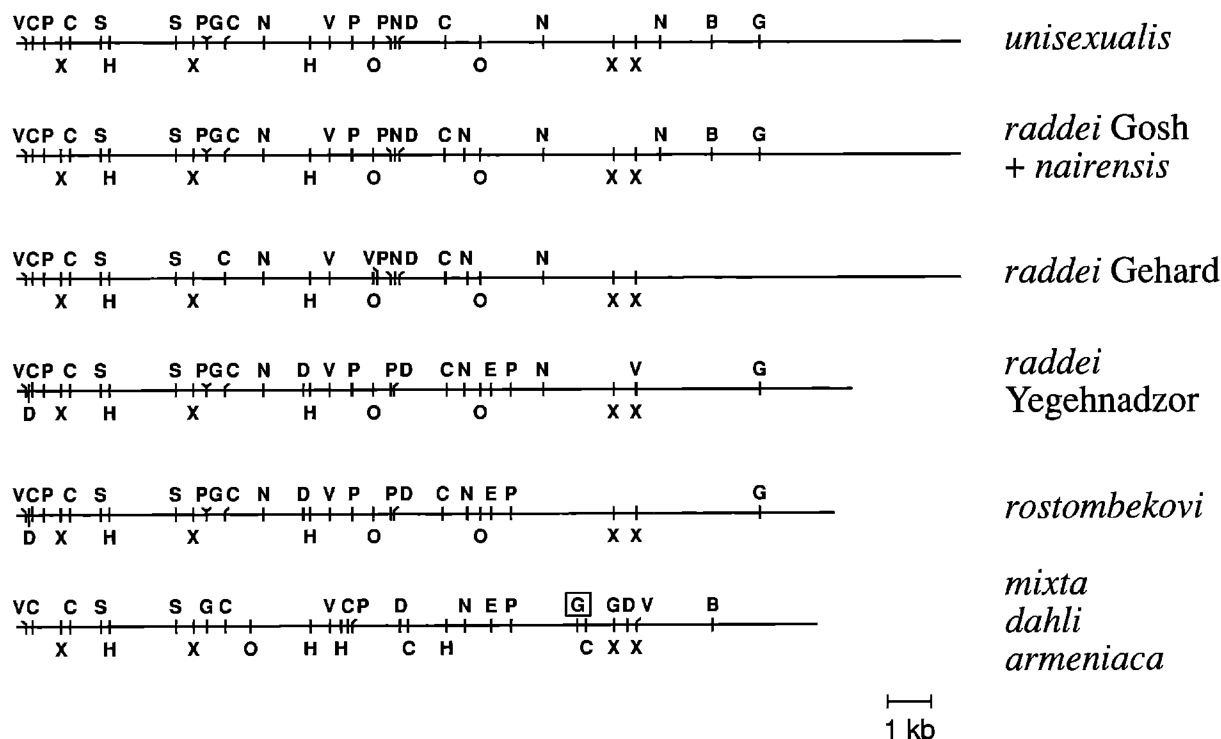

Fig. 2. Linearized and aligned maps of 6bp cleavage sites in mtDNAs from parthenogenetic and bisexual species of *Lacerta*. The [G] in the bottom map indicates a *Bgl*II site present in *L. mixta*, but not *L. dahli* or *L. armeniaca*. Large scale length variation is obvious at the right hand end of the maps. The 200 bp addition in the mtDNA of *L. raddei* from Gosh was omitted to facilitate alignment. Abbreviations: B = *Bam*HI, C = *Bcl*I, D = *Spe*I, E = *Eco*RI, G = *Bgl*II, H = *Hind*III, N = *Nhe*I, O = *Nco*I, P = *Pvu*II, S = *Sac*II, V = *Eco*RV, and X = *Xba*I.

Table 2. Estimates of sequence divergence (δ) between representatives of parthenogenetic species of *Lacerta* and their bisexual relatives. Estimates identified by a superscript asterisk are approximations based on proportions of shared fragments (see text). Other values are derived from comparisons of mapped cleavage sites (Fig. 2). mtDNAs most similar to those from the parthenogens are in bold type.

	Parthenogenetic species			
Sexual species	*unisexualis*	*uzzelli*	*dahli/armeniaca*	*rostombekovi*
nairensis	**0.003**	0.096	0.084	0.033
raddei – Gosh	**0.003**	0.096	0.084	0.033
- Gehard	0.014	-	0.090	0.040
- Yegehnadzor	0.036	-	0.086	**0.006**
mixta	0.095	-	**0.003**	0.093
valentini	0.094*	**0.000***	0.116*	-
portschinskii	-	-	0.092*	0.070*
parvula	-	0.094*	-	-

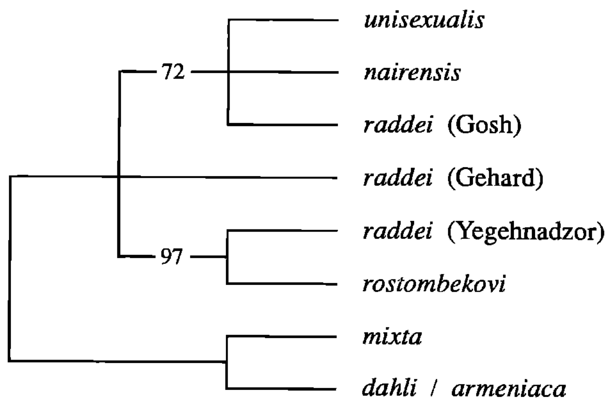

Fig. 3. Phylogeny of mapped mtDNAs from parthenogenetic and sexual species of *Lacerta*. The phylogeny shown is a consensus of 100 bootstrap pseudoreplicates, each using the Exhaustive Search option of PAUP 3.0i and Dollo parsimony. The values on internal branches are the number of boostrap replicates (out of 100) in which the group to the right was formed. The shortest trees have 32 steps for 27 informative characters, a consistency index of 0.84 and a retention index of 0.95. The branch uniting *L. unisexualis, L. nairensis* and the Gosh population of *L. raddei* was defined by 1-2 restriction sites, and the branch uniting the Yegehnadzor population of *L. raddei* and *L. rostombekovi* by 6 characters. If the 2.5 kb insertion was included as a character, the Gehard population of *L. raddei* grouped with the *L. unisexualis et al.* clade in 65% of bootstraps.

Results

Length variation. Preliminary screening of the samples with the 6-bp recognizing enzyme *Bcl*I revealed between two and seven fragments per genome. There was no variation within any parthenogenetic species, but substantial differences resulting from both site changes and length mutations were observed within and among the sexual species.

The size of the mtDNA (estimated from *Bcl*I digests) varied from 17.3 kb in *L. parvula* to 21.7 kb in *L. raddei* from Gosh (Table 1). Although there is minor variation (< 200 bp) within and between individuals, the mtDNA genomes do not form a size continuum. Most genomes are between 17.3 and 18.5 kb, but mtDNAs from *L. unisexualis, L. nairensis* and the Gehard population of *L. raddei* were 21.5 kb and those from the Gosh population of *L. raddei* were 21.7 kb (Table 1). Comparison of the cleavage site maps (Fig. 2) indicates that the length variation is mostly restricted to a single region devoid of restriction sites (Fig. 2), and approximately 2 kb from the 1.7 kb SacII fragment located in the ribosomal genes (Carr, Brothers & Wilson, 1987). This placement suggests that the length variation occurs in or near the non-coding region, as in other vertebrates (Moritz, Dowling & Brown, 1987).

Comparisons between sexual and parthenogenetic species. The 13 6-bp recognizing enzymes cleaved at between 26 and 31 sites per sample. The enzyme *Sal*I did not cleave any of the mtDNAs; *Sac*II revealed the same fragment pattern (> 15 kb and 1.7 kb) in all samples. The other 11 enzymes each revealed variation resulting from the gain or loss of cleavage sites. The estimates of sequence divergence between mtDNAs of the parthenogenetic species and the sexual species implicated in their

ancestry permit an unambiguous assignment of maternal parent for four of the five parthenogenetic species (Table 2).

mtDNA from the parthenogenetic species *L. rostombekovi* is very distinct from that of *L. portschinskii* (F = 0.27, δ = 7.9%), one of its parental species, but very similar to mtDNA from the Yegehnadzor population of *L. raddei*, differing from it by only two cleavage sites (δ = 0.6%). In comparison to mtDNA from *L. rostombekovi*, those from other populations of *L. raddei* were more divergent in restriction sites (Table 2), were longer (Fig. 2) and were distinct in the phylogenetic analysis (Fig. 3).

mtDNAs from the parthenogenetic species *L. dahli* and *L. armeniaca* are identical to each other at the 26 sites assayed. These two share *L. mixta* as a parental species (Uzzell & Darevsky, 1975), and their mtDNA differs from that of *L. mixta* by a single site loss (δ = 0.3%). *L. dahli* shares few mtDNA fragments with its other sexual parent, *L. portschinskii* (F = 0.22, δ = 9.2%), and *L. armeniaca* even fewer with its other parent, *L. valentini* (F = 0.15, δ = 11.6%).

mtDNAs of *L. unisexualis* and one of its parental species, *L. valentini*, share few fragments (F = 0.21, δ = 9.4%), indicating that *L. valentini* is not the maternal parent species of *L. unisexualis*. It is not clear, however, whether the mtDNA from *L. nairensis* (the other putative parent, Uzzell & Darevsky, 1975) or from *L. raddei* is most closely related to that of *L. unisexualis*. mtDNAs from *L. nairensis* and the Gosh population of *L. raddei* were identical at the 29 6-bp sites assayed, but differed in length, the Gosh sample being 200 bp longer. The mtDNAs of *L. unisexualis* from Kars and Ankavan were identical and lacked and *Nhe*I site present in *L. nairensis* and *L. raddei* (Gosh). The *L. unisexualis* samples were the same length as those from *L. nairensis*. The mtDNA of *L. unisexualis* thus resembles that of *L. nairensis* slightly more than that of *L. raddei* from Gosh. However, phylogenetic analysis, which exludes unique and constant sites (18 of 45 sites) and length variants, does not resolve which of the two sexual species is the most likely maternal parent of *L. unisexualis* (Fig. 3). Comparisons of the fragment patterns generated by 4-bp recognizing enzymes revealed slightly higher divergences, but did not clarify the situation. Again, the samples from *L. nairensis* and *L. raddei* (Gosh) were very similar (δ = 0.5%) and were virtually equidistant from the *L. unisexualis* mtDNA (*L. nairensis*, δ = 0.94%; *L. raddei*, δ = 0.99%).

Table 3. Estimates of mtDNA nucleotide diversity (π, Nei & Li, 1979) within and between populations of parthenogenetic and bisexual *Lacerta*. The among-population diversity for *L. nairensis* is based on the propertion of shared fragments, others on shared sites. Indiv. = individuals; loc. = localities; pop. = populations.

Species	Sample Indiv.	size Locs.	No. types	Nucleotide Within pop.	Diversity Among pop.
Parthenogenetic:					
unisexualis	14	2	1	0.000	0.000
dahli	17	2	1	0.000	0.000
armeniaca	20	3	2	0.0002	0.000
Sexual:					
mixta	8	2	4	0.0004	0.0004
nairensis	4	2	4	0.003	0.018
raddei	8	3	3	0.000	0.031

For *L. uzzelli*, few mtDNA fragments are shared between *L. uzzelli* and either of the parental species suggested by Darevsky & Danielyan (1977) (*L. parvula*, F = 0.21, δ = 9.4%; *L. nairensis,* F = 0.22, δ = 9.2%). However, the mtDNAs from *L. uzzelli* and *L. valentini* were identical at all 27 sites assayed (δ = 0.0%).

Variation within sexual and parthenogenetic species. Variation within the parthenogenetic species *L. unisexualis, L. dahli*, and *L. armeniaca* and within the sexual species *L. mixta, L. nairensis*, and *L. raddei* was assessed by comparing samples from the same and geographically separated populations with six 4-bp recognizing enzymes (Table 3). These enzymes assayed between 109 and 121 sites per individual. No variation attributable to the gain or loss of cleavage sites was found among the 14 specimens of *L. unisexualis* from three localities. The samples of *L. dahli* (N = 17), which included both 'yellow' and 'common' morphological variants (Darevsky, 1967), were uniform except for minor length variation. All but one of the mtDNAs from *L. armeniaca* (N = 20) were identical to the samples of *L. dahli*. The single variant, from Hrazdan (Fig. 1), differed in having one additional *Mbo*I site and an extra *Taq*I site, corresponding to an estimated sequence divergence of 0.2%. Two

specimens of a morphologically distinct form of *L. armeniaca* (I. S. Darevsky, unpubl. data) had the common type of mtDNA. Nucleotide diversity in *L. armeniaca* was 0.02% and was zero in the other parthenogenetic species.

Analysis of samples of *L. raddei* from three localities (Table 1, Fig. 1) revealed uniformity within localities, but no sharing of mtDNA types between localities (Table 3). The complex differences between the fragment patterns generated by the 4-bp enzymes made it difficult to estimate sequence divergence between localities (this technique usually being limited to mtDNAs with $\delta < 2\%$, Dowling, Moritz & Palmer, 1990). Comparisons of 6-bp cleavage site maps for *L. raddei* from different localities (Fig. 2) produced estimates of sequence divergence ranging from 2.5% to 3.9% (mean = 3.1%). A survey of four *L. nairensis* from two localities revealed that each sample was distinct. The three samples from Sevan differed by between one and three site changes, corresponding to sequence divergences of between 0.1 and 0.4% (mean = 0.27%). Comparing the samples from Hrazdan and Sevan, the fragment changes were too complex for individual site changes to be inferred. Based on the proportion of shared fragments (F = 0.80), they differ by approximately 1.8%.

Analogous surveys of *L. mixta*, the maternal sexual parent of *L. dahli* and *L. armeniaca*, also revealed variation within and between two populations (Table 3). Four mtDNA types were found, each distinguished by a single site change. The mean sequence divergence among these variants is 0.15%. A mtDNA identical to the common type in *L. dahli* and *L. armeniaca* was found among *L. mixta* from both localities sampled.

Discussion

Maternal ancestry. The mtDNA comparisons unambiguously identify the maternal parent of all parthenogenetic species of *Lacerta* except for *L. unisexualis* (Fig. 4). The mtDNAs from *L. uzzelli* and *L. valentini* were identical, indicating that the latter provided the maternal parent. Although there are minor differences in the 6-bp site comparisons, it is also clear that *L. mixta* provided the maternal parents of both *L. dahli* and *L. armeniaca*, and that the maternal parent of *L. rostombekovi* came from *L. raddei*.

For *L. unisexualis*, the mtDNA restriction site data alone do not discriminate between *L. nairensis* and the Gosh population of *L. raddei* as the maternal parent. The survey of variation at four protein coding loci (Uzzell & Darevsky, 1975) also failed to discriminate between *L. nairensis* and *L. raddei*, although the former was suggested as being the more likely parent. This receives some support from the mtDNA evidence as the Gosh sample of *L. raddei* was 200 bp longer that than of *L. nairensis* or *L. unisexualis* (Fig. 2).

The difficulty in assigning the maternal parentage of *L. unisexualis* emphasizes the close relationships and genetic similarity of *L. raddei* and *L. nairensis*. These are now recognised as separate species because they maintain differences in morphology and mating behaviour in sympatry (Fig. 1; J. Eiselt, I. S. Darevsky & J. Schmidtler, in preparation). The mtDNA variation indicates paraphyletic relationships within *L. raddei*, with the Gosh population being more closely related to the sample of *L. nairensis* than to conspecific samples from Yegehnadzor and Gehard (Fig. 3). While this paraphyly could be a result of random sorting of ancestral polymorphism (Neigel & Avise, 1985), it emphasizes the need for further analysis of mtDNA variation and relationships within and between these two species.

These assignments of maternal ancestry are consistent with the parental pairs assigned by Uzzell and Darevsky (1975), but implicate *L. valentini* in the ancestry of *L. uzzelli* (contra Darevsky & Danielyan, 1977). The assignments (Fig. 4) are also

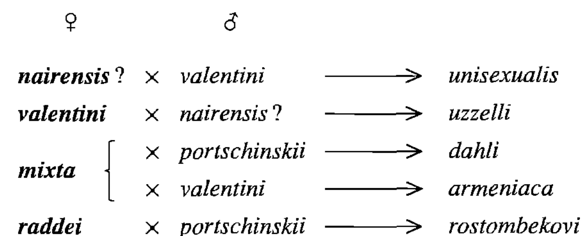

Fig. 4. Summary of proposed origins if parthenogenetic species of *Lacerta* based on the evidence from mtDNA analysis in combination with analyses of allozymes, morphology, and distributions (Uzzell & Darevsky, 1975; Darevsky & Danielyan, 1977). The question mark after *nairensis* indicates that it is still not certain whether this species or a population of *L. raddei* is involved.

consistent with predictions made from the distribution of sex chromosomes (Darevsky, Kupriyanova & Danielyan, 1986; Kupriyanova, 1989, unpubl. data). Females of both *L. raddei* and *L. nairensis* have a small heterochromatic sex (W) chromosome that also appears in *L. rostombekovi* and *L. unisexualis*. Similarly, the distinctive W chromosome of *L. mixta* is seen in both *L. armeniaca* and *L. dahli* (Kupriyanova, unpubl. data), indicating that *L. mixta* acted as their maternal sexual parent.

Evidence for recent origins. On the basis of distributions around Lake Sevan, Uzzell and Darevsky (1975) suggested that that both *L. unisexualis* and *L. rostombekovi* were at least 5000 years old. The maximum age was unclear, but the authors speculated that the parthenogenetic species arose after the Würm glaciations (10,000 years ago). The low mtDNA divergences between the parthenogenetic species and their respective maternal sexual parents provides strong evidence that the parthenogenetic species arose recently. Levels of divergence between the parthenogenetic species and their maternal sexual ancestor species vary from zero (*L. valentini* vs. *L. uzzelli*) to 0.6% (*L. raddei* vs. *L. rostombekovi*). This is considerably less than divergences estimated among populations of *L. raddei* (Table 3). Unless the rate of mtDNA evolution within the parthenogenetic species has slowed dramatically, they must have arisen recently, possibly within the past few tens of thousands of years.

Indications of founder events. The parthenogenetic species *L. unisexualis, L. dahli* and *L. armeniaca* have exceptionally low mtDNA nucleotide diversities, even for parthenogenetic vertebrates (cf. Moritz, 1991: Table 1). This lack of variation suggests that each of these lineages has been through a stage with very small population size, presumably a founder event at the origin. For *L. unisexualis*, a strong founder event is consistent with the results of skin grafting experiments that revealed isogenicity among samples from widely separated localities in Armenia (Darevsky & Danielyan, 1979).

The identity of mtDNAs from *L. dahli* and *L. armeniaca* reflect an unusual pair of events in which the females involved in different hybridizations giving rise to distinct parthenogenetic species (Fig. 4) had indistinguishable mtDNAs. The evidently close matrilineal relationship of their maternal founders suggests that the origins of *L. dahli* and *L. armeniaca* were close both in time and space. A similar result was reported for the parthenogenetic species *Cnemidophorus tesselatus* and *C. neomexicanus* which have very similar mtDNAs derived from *C. marmoratus* (Densmore, Wright & Brown, 1989).

Where there is substantial geographically structured variation among populations of the sexual maternal ancestor species, uniformity of mtDNA in a parthenogenetic species indicates that it probably arose in a restricted geographic area (Moritz *et al.*, 1989a). This is almost certainly the case for *L. unisexualis*. There is marked differentiation among populations of the maternal species, whether *L. nairensis* or *L. raddei*, but none among populations of *L. unisexualis*. The evidence for geographic restriction in the origin of *L. dahli* and *L. armeniaca* is weaker. Their maternal ancestor, *L. mixta*, showed little variation among populations, with mtDNAs indistinguishable from those in the parthenogenetic species being found in both localities. These two localities were only ca. 30 km apart, however, and further sampling is necessary to assess geographic variation across the range of *L. mixta*. Further sampling is also required to assess variation within the parthenogenetic species *L. rostombekovi* and *L. uzzelli*.

At least for the species having *L. raddei* or *L. nairensis* as a maternal ancestor, further comparison of mtDNA from the parthenogenetic species with geographic variants of the sexuals may provide information about the geographic history of these forms. For example, the close relationship between the mtDNAs from *L. rostombekovi* and the Yegehnadzor sample of *L. raddei* could indicate that this species arose to the south of Lake Sevan, far from any extant population of the other parent, *L. portschinskii*, rather than in the north (e.g., in the vicinity of Gosh; Fig. 1) where *L. portschinskii* and *L. raddei* are currently sympatric and form hybrids (Uzzell & Darevsky, 1973; I. S. Darevsky & F. Danielyan, unpubl. data). However, it is clear that the sexual species have changed their ranges because not all parental pairs are presently sympatric (compare Figs. 1 and 4).

General features of parthenogenesis in lizards. The mtDNA evidence for *Lacerta* extends the generality of two conclusions drawn from genetic analyses

of other parthenogenetic lizards, all of which have low mtDNA diversity relative to sexual congeners (reviewed by Moritz *et al.*, 1989b. Moritz, 1991). These conclusions are (i) that in each case the transition from sexual reproduction to parthenogenesis results from limited hybridizations involving closely related females, and (ii) that parthenogenetic taxa are of recent origin. The consistency in these patterns is remarkable given that they are repeated across four continents (Eurasia, North and South America, Australia) and among lizard species living in a wide variety of habitats (riparian, arid scrubland, montane, and oceanic island).

The nature of the founder event in the origin of these lineages varies. In some taxa, such as *H. binoei* (Moritz *et al.*, 1989b), *C. tesselatus* (Parker & Selander, 1976) and *C. lemniscatus* (Sites *et al.*, 1990), high levels of genetic diversity in allozymes or chromosomes combined with low variation in mtDNA suggests multiple origins from a geographically restricted sample of females (Densmore, Wright & Brown, 1989; Vyas *et al.*, 1990; Moritz, 1991). In other cases (e.g., *C. neomexicanus*, Parker & Selander, 1984; Densmore, Wright & Brown, 1989) in which there is low diversity for allozymes as well as mtDNA, the origins appear to have been strongly restricted both numerically and geographically. Surveys of allozyme variation within parthenogenetic species of *Lacerta* are necessary to determine into which category they fall.

These indications of recent origins and strong founder events are consistent with the notion that there are strong constraints on the origin and persistence of parthenogenetic lineages (Williams, 1975; Moritz *et al.*, 1989a; Vrijenhoek, 1989). The appropriate question may not be 'why are alternatives to sexual reproduction so rare', but 'why do they exist at all?'

Acknowledgements

This research was supported by grants from the National Science Foundation (USA) to T. Uzzell (DEB 77-13472, 81-11397) and to W. M. Brown and C. Moritz (85-17830), from the National Geographic Society (2804-84) to T. Uzzell and H. Hotz, and from the Australian Research Council to C. Moritz. Thanks to S. Lavery and A. Heideman for laboratory assistance. Thanks also to D. B. Wake and T. Papenfuss for their hospitality to Moritz and Darevsky.

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