Allozyme variation in populations of *Lacerta raddei* and *Lacerta nairensis* (Sauria: Lacertidae) from Armenia

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Abstract. *Lacerta raddei* and *Lacerta nairensis* have been recognized as two separate species based on morphology and behavior, and each has been implicated as a sexual parent of different parthenogenetic forms. However, recent mitochondrial DNA work failed to distinguish these two as separate species. We examined genetic diversity at 36 allozyme loci from six populations of *L. nairensis* and four populations of *L. raddei*. There were no fixed allelic differences between the two. Mean heterozygosity was slightly higher among populations of *L. raddei* than among populations of *L. nairensis*. A Distance Wagner phenogram showed that the northernmost population of *L. raddei* clustered with the *L. nairensis* populations; the other *L. raddei* populations clustered together. We suggest that *L. raddei* and *L. nairensis* may not be separate species, a finding which has important implications for determining the origins of some parthenogenetic *Lacerta*.

Introduction

Caucasian rock lizards of the genus *Lacerta* include several parthenogenetic taxa which, based on chromosomal and molecular evidence, are thought to have arisen from hybridization between various bisexual species (Darevsky, 1967; Darevsky et al., 1985; Moritz et al., 1992). *Lacerta raddei* and *L. nairensis* have been implicated as maternal parents of the parthenogenetic *L. rostombekovi* and *L. unisexualis*, respectively (Uzzell and Darevsky, 1975); *L. nairensis* is also possibly the paternal parent of *L. uzzelli* (Darevsky and Danielyan, 1977; Moritz et al., 1992). Interestingly, sterile triploids have resulted from crosses between males of bisexuals and females of sympatric parthenogens, either *L. armeniaca* or *L. unisexualis* in the case of *L. nairensis*, or *L. rostombekovi* in the case of *L. raddei* (Darevsky, 1967; Darevsky et al., 1986).

Lacerta nairensis had been considered a subspecies of *L. raddei* (Uzzell and Darevsky, 1973), but work on morphology and behavior by Darevsky (1967) and Eiselt et al. (1993; in prep., as cited in Moritz et al., 1992) shows that the two may be distinct species. Based on mitochondrial DNA variation, Moritz et al. (1992) confirmed that *L. raddei* was the maternal parent of *L. rostombekovi*, but they also found that at least one population of *L. raddei* could not be considered distinct from *L. nairensis*. Consequently, either species could be the maternal parent of *L. unisexualis*.

This study is part of a larger investigation examining allozyme variation and phylogeny among bisexual (Murphy et al., 1996a) and parthenogenetic species of the subgenus *Archaeolacerta*. We looked at allozyme variation to determine the genetic variability among several populations of *L. raddei* and *L. nairensis*, including the *L. raddei* populations examined by Moritz et al. (1992), to investigate (a) whether or not they can be considered distinct species, and if so (b) which is the maternal parent of the parthenogen, *L. unisexualis*.

Materials and methods

Specimens of *Lacerta raddei* and *L. nairensis* were collected from several populations in central Armenia, near Lake Sevan, in May 1993 (see Appendix for locality data, sample sizes, and voucher numbers; fig. 1). *Lacerta nairensis* collected on the north slope of Adis Mountain in the Gegam Range (population Adis 1) were considered to be from a different population than *L. nairensis* from the south slope (population Adis 2).

The lizards were euthanized with an overdose of sodium pentobarbitol. Samples of liver, heart, and tail muscle were dissected from each individual and frozen in liquid nitrogen. In some cases, specimens were frozen whole and dissected upon return to the Royal Ontario Museum (ROM). Collection and euthanasia of specimens was carried out in accordance with CCAC guidelines (Canadian Council on Animal Care, 1984), under approved protocols to RWM. All voucher specimens have been catalogued and deposited in the ROM herpetology collection.

A mix of heart, liver, and muscle samples for each individual was homogenized and used for starch gel electrophoresis on 11% gels. All electrophoresis procedures, staining recipes, and enzyme and locus nomenclature follow Murphy et al. (1996b). We were able to resolve 36 loci, and wherever possible, allozyme loci were resolved using two buffer systems to uncover hidden variation (table 1).

Allozyme data were analyzed using BIOSYS-1 (Version 1.7; Swofford and Selander, 1989). The percentage of loci polymorphic, mean number of alleles per locus, and the mean heterozygosity (direct-count) were calculated for each population, and conformity to Hardy-Weinberg expectations was calculated by Chi-square using Levene's (1949) correction factor for small sample sizes where appropriate. Interpopulation allozyme frequency heterogeneity was estimated using Wright's hierarchical F-statistics (Wright, 1965, 1978) and a contingency Chi-square test (Workman and Niswander, 1970). The

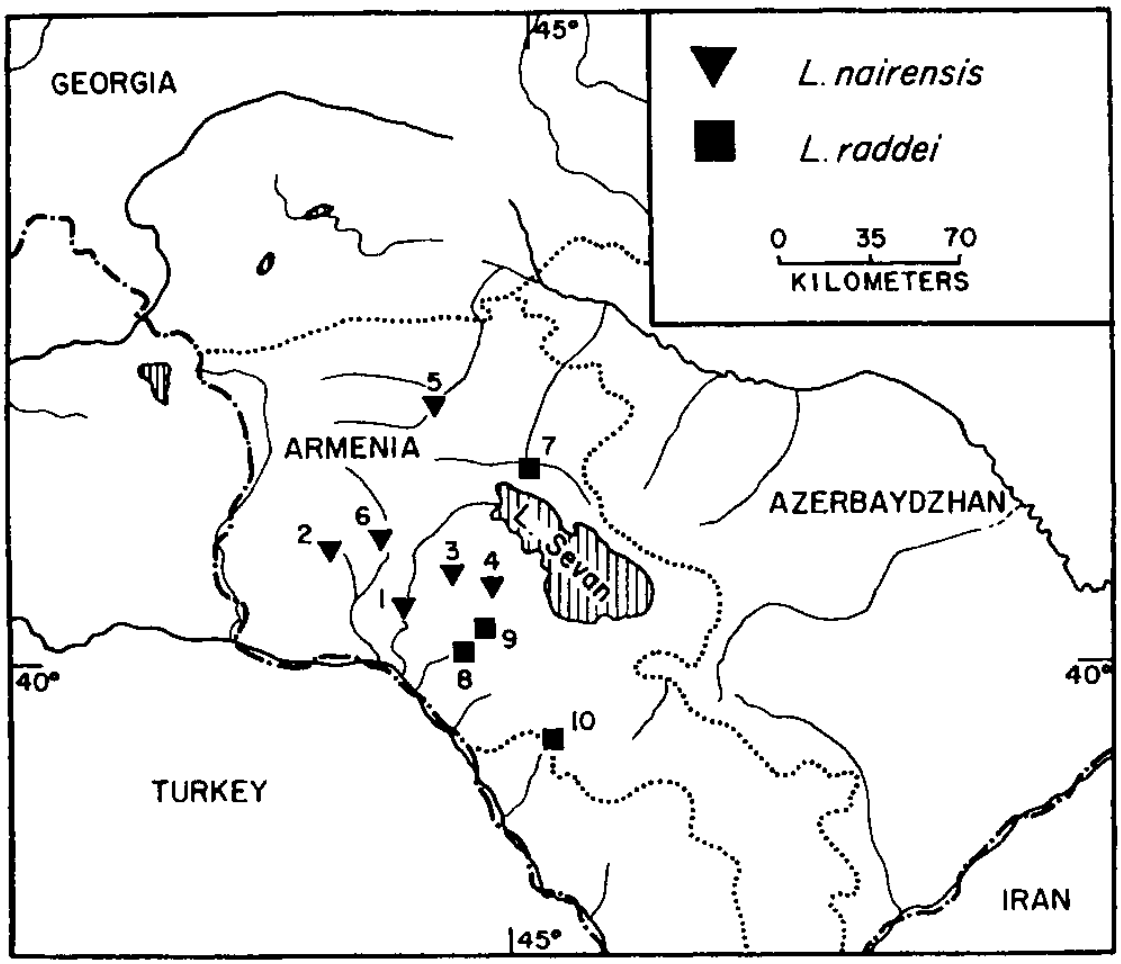

Figure 1. Map of locations in Armenia where lizards were collected: *Lacerta nairensis* (1 = Yerevan, 2 = Aragatz, 3 = Adis 1, 4 = Adis 2, 5 = Tumanyan, 6 = Apnaguch) and *Lacerta raddei* (7 = Gosh, 8 = Chosrov, 9 = Geghart, 10 = Yekhegnadzor).

genetic divergence among the populations was examined with the genetic distance coefficients of Rogers (1972) and Nei (1978); the Rogers' distances were then clustered by the Distance Wagner procedure (Farris, 1972).

Results

All populations were monoallelic at 16 of the 36 loci resolved: mAat-A, Ada-A, Cbp-1, Gda-A, βGlur-A, Gpi-B, Gtdh-A, G6pdh-A, Ldh-B, mMdh-A, sMdhp-A, mMdhp-A, Mpi-A, Pgk-A, Pk-A, and mSod-A. The populations of *Lacerta nairensis* were monoallelic for five additional loci (mAcoh-A, Ck-A, Ck-C, Gcdh-A and Gsr-1) and the populations of *L. raddei* were monoallelic for another five loci (βGa-1, mIdh-A, Pgm-A, sSod-A, and Tpi-A). For several polymorphic loci, the least common allele occurred

Table 1. Names and Enzyme Commission Numbers of enzyme systems analyzed and the buffer systems used in analysis of 36 loci in *Lacerta raddei* and *Lacerta nairensis*.

Enzyme Name	Abbreviation (# loci scored)	EC Number	Buffer[a]
N-Acetyl-β-glucosaminidase	βGA (1)	EC 3.2.1.30	2, 6
Acid Phosphatase	ACP-B (1)	EC 3.1.3.2	1
Aconitase Hydratase	ACOH (2)	EC 4.2.1.3	3, 4
Adenosine Deaminase	ADA (1)	EC 3.5.4.4	6
Aspartate Aminotransferase	AAT (2)	EC 2.6.1.1	1, 2
Catalase	CAT (1)	EC 1.11.1.6	3, 7
Calcium-Binding Proteins	CBP (1)	Nonspecific	2, 6
Creatine Kinase	CK (2)	EC 2.7.3.2	4, 5
"Esterase-D"	EST-D (1)	EC 3.1.1.-	6, 7
Glucose Dehydrogenase	GCDH (1)	EC 1.1.1.118	4, 5
Glucose-6-phosphate Dehydrogenase	G6PDH (1)	EC 1.1.1.49	1, 7
Glucose-6-phosphate Isomerase	GPI (1)	EC 5.3.1.9	4, 7
β-Glucosidase	βGLUS (1)	EC 3.2.1.21	6
β-Glucuronidase	βGLUR (1)	EC 3.2.1.31	6
Glutamate Dehydrogenase	GTDH (1)	EC 1.4.1.2	6
Glutathione Reductase	GSR (1)	EC 1.6.4.2	1
Guanine Deaminase	GDA (1)	EC 3.5.4.3	1, 6
Isocitrate Dehydrogenase	IDH (2)	EC 1.1.1.42	1, 2
L-Lactate Dehydrogenase	LDH (2)	EC 1.1.1.27	4, 5
Malate Dehydrogenase	MDH (1)	EC 1.1.1.37	1
Malate Dehydrogenase ($NADP^+$)	MDHP (2)	EC 1.1.1.40	4, 6
Mannose-6-phosphate Isomerase	MPI (1)	EC 5.3.1.8	2
Peptidase-B	PEP-B (1)	EC 3.4.-.-	2, 3
Phosphoglucomutase	PGM (1)	EC 5.4.2.2	1, 2, 5
Phosphoglycerate Kinase	PGK (1)	EC 2.7.2.3	4
Purine-nucleoside Phosphorylase	PNP (1)	EC 2.4.2.1	1, 2
Pyruvate Kinase	PK (1)	EC 2.7.1.40	3, 4
Superoxide Dismutase	SOD (2)	EC 1.15.1.1	5, 7
Triose-phosphate Isomerase	TPI (1)	EC 5.3.1.1	5

[a] 1 = Amine-citrate morpholine, pH 6.1; 2 = Amine-citrate morpholine, pH 7.5; 3 = Tris-citrate, pH 7.0; 4 = Tris-citrate, pH 8.0; 5 = Tris-citrate/borate, pH 8.7; 6 = Tris-HCl, pH 8.2; 7 = Tris-borate EDTA, pH 8.6.

in only one individual (table 2); for example, in the population of *L. nairensis* from Apnaguch, one individual (ROM 24844) exhibited rare alleles for four loci (sAat-A, Cat-A, Pnp-A and sSod-A). For some loci, only one population exhibited any variation: *L. nairensis* from Yerevan at Pgm-A, and from Aragatz at βGa-1 and Tpi-A; *L. raddei* from Gosh at mAcoh-A, Ck-C and Gcdh-A, and from Chosrov at Ldh-A. Some alleles

Table 2. Genotype frequencies and sample sizes (in brackets) for polymorphic loci in populations of *Lacerta nairensis* (1-6) and *Lacerta raddei* (7-10); m = mitochondrial locus; s = supernatant locus. Populations: 1 = Yerevan, 2 = Aragatz, 3 = Adis 1, 4 = Adis 2, 5 = Tumanyan, 6 = Apnaguch, 7 = Gosh, 8 = Chosrov, 9 = Geghart, 10 = Yekhegnadzor.

Population	1	2	3	4	5	6	7	8	9	10
Locus										
sAat-A	aa(33)	aa(30)	aa(3)	aa(5)	aa(19)	aa(11) bb(1)	aa(29) ac(1)	aa(30)	aa(30)	aa(3)
sAcoh-A	aa(33)	aa(30)	aa(3)	aa(5)	aa(19)	aa(10) ac(1) cc(1)	aa(21) ac(7) cc(1) ae(1)	aa(28) ac(2)	aa(30)	aa(1) ac(1) cc(1)
mAcoh-A	aa(33)	aa(30)	aa(3)	aa(5)	aa(19)	aa(12)	aa(29) ac(1)	aa(30)	aa(30)	aa(3)
Acp-B	aa(32)	aa(30)	aa(3)	aa(1) ac(1) cc(3)	aa(19)	aa(12)	aa(29) ab(1)	aa(30)	aa(30)	aa(3)
Cat-A	aa(33)	aa(29) bb(1)	aa(3)	aa(4)	aa(19)	aa(9) ac(1)	aa(29) ab(1)	aa(30)	aa(29) ab(1)	aa(3)
Ck-A	aa(33)	aa(30)	aa(3)	aa(5)	aa(19)	aa(12)	aa(29) ab(1)	aa(29) ab(1)	aa(30)	aa(3)
Ck-C	aa(33)	aa(30)	aa(3)	aa(5)	aa(19)	aa(12)	aa(29) ab(1)	aa(30)	aa(30)	aa(3)
Est-D	aa(33)	aa(23) ab(4) bb(2) ac(1)	aa(3)	aa(5)	aa(19)	aa(12)	aa(25) ab(5)	aa(20) ab(5) bb(1) af(1) dd(2) ee(1)	aa(26) ab(4)	aa(2) bb(1)
βGa-1	aa(33)	aa(10) bb(20)	aa(3)	aa(5)	aa(19)	aa(12)	aa(30)	aa(30)	aa(30)	aa(3)
Gcdh-A	aa(33)	aa(30)	aa(3)	aa(5)	aa(19)	aa(12)	aa(28) bb(1) cc(1)	aa(30)	aa(30)	aa(3)
βGlus-A	aa(33)	aa(21) ab(2)	aa(3)	aa(5)	aa(19)	aa(12)	aa(30)	bb(29) bf(1)	aa(21) ab(3) bb(2) ac(4)	bb(3)
Gsr-1	aa(33)	aa(30)	aa(3)	aa(5)	aa(19)	aa(12)	aa(28) ab(1)	aa(29) ab(1)	aa(30)	aa(3)
sIdh-A	aa(33)	aa(30)	aa(3)	aa(5)	aa(19)	aa(10) ac(1) cc(1)	aa(30)	aa(29) ab(1)	aa(30)	aa(3)
mIdh-A	aa(33)	aa(29) ab(1)	aa(2) ab(1)	aa(4)	aa(19)	aa(12)	aa(30)	aa(30)	aa(30)	aa(3)
Ldh-A	aa(33)	aa(30)	aa(3)	aa(5)	aa(19)	aa(12)	aa(30)	aa(28) ab(1) bb(1)	aa(30)	aa(3)

Table 2. Continued

Population	1	2	3	4	5	6	7	8	9	10
Pep-B	aa(32) ab(1)	aa(30)	aa(3)	aa(5)	aa(19)	aa(12)	aa(30)	aa(15) ab(1) bb(1) ac(10) cc(3)	aa(17) ab(2) bb(1) ac(10)	aa(2) bb(1)
Pgm-A	aa(31) ab(1) bb(1)	aa(30)	aa(3)	aa(5)	ab(2) bb(17)	aa(12)	aa(30)	aa(30)	aa(30)	aa(3)
Pnp-A	aa(33)	aa(30)	aa(3)	aa(5)	aa(15) ac(4)	aa(11) dd(1)	aa(30)	aa(30)	bb(1) bc(2) cc(27)	aa(3)
sSod-A	aa(33)	aa(30)	aa(3)	aa(4)	aa(19)	aa(11) bb(1)	aa(30)	aa(30)	aa(30)	aa(3)
Tpi-A	aa(33)	aa(29) ab(1)	aa(3)	aa(5)	aa(19)	aa(12)	aa(30)	aa(30)	aa(30)	aa(3)
PLP[a](95%)	0.00	5.86	2.78	2.78	5.56	16.67	8.33	8.33	11.11	8.33
PLP[a]	5.56	16.67	2.78	2.78	5.56	16.67	27.78	22.22	13.89	8.33
MNA[b]	1.06	1.19	1.03	1.03	1.06	1.17	1.33	1.33	1.19	1.08
(±SE)	(0.04)	(0.08)	(0.03)	(0.03)	(0.04)	(0.06)	(0.10)	(0.13)	(0.09)	(0.05)
MHD[c]	0.002	0.009	0.009	0.006	0.009	0.007	0.019	0.022	0.024	0.009
(±SE)	(0.001)	(0.005)	(0.009)	(0.006)	(0.006)	(0.004)	(0.009)	(0.012)	(0.013)	(0.009)

[a] percentage of loci polymorphic (0.95 criterion and no criterion)
[b] mean number of alleles per locus (±standard error)
[c] mean heterozygosity by direct count (±standard error)

were relatively common in one population but rare or non-existent in the other populations, for example, Acp-B (c) in Adis 2, βGa-1 (b) in Aragatz, and Pnp-A (c) in Geghart. βGlus-A (a) was absent in the populations of *L. raddei* from Chosrov and Yekhegnadzor, yet common in all the other populations. However, there were no fixed allelic differences between *L. raddei* and *L. nairensis*.

For the populations of *L. nairensis*, the most variable loci were Cat-A, mIdh-A, Pgm-A and Pnp-A. For *L. raddei*, sAcoh-A, Est-D and Pep-B were the most variable. The allele frequencies at eight loci did not conform to Hardy-Weinberg expectations, even if uncommon genotypes were pooled and exact probabilities calculated: for *L. nairensis* from Yerevan, Pgm-A ($\chi^2 = 20.995$, df = 1, $P = 0.000$; exact probability $P = 0.046$); from Aragatz, Cat-A ($\chi^2 = 59.018$, df = 1, $P = 0.000$; exact $P = 0.017$) and βGa-1 ($\chi^2 = 31.309$, df = 1, $P = 0.000$; exact $P = 0.000$); from Apnaguch, sAat-A, Pnp-A and sSod-A (for each, $\chi^2 = 23.048$, df = 1, $P = 0.000$; exact $P = 0.043$); for *L. raddei* from Gosh, Gcdh-A ($\chi^2 = 118.036$, df = 3, $P = 0.000$; exact probability after pooling, $P = 0.001$); from Chosrov, Est-D ($\chi^2 = 99.808$, df = 10, $P = 0.000$; exact probability after pooling, $P = 0.025$).

Table 3. Contingency Chi-square values for loci showing allele frequency heterogeneity, all populations combined.

Locus	No. of alleles	Chi-square	D. F.	*P*
sAat-A	3	36.162	18	.00673
sAcoh-A	3	66.927	18	.00000
mAcoh-A	2	5.514	9	.78739
Acp-B	3	274.918	18	.00000
Cat-A	3	23.886	18	.15880
Ck-A	2	4.523	9	.87374
Ck-C	2	5.514	9	.78739
Est-D	6	69.789	45	.01036
Gcdh-A	3	22.228	18	.22202
βGlus-A	4	347.404	27	.00000
Gsr-I	2	4.602	9	.86754
βGa-1	2	245.143	9	.00000
sIdh-A	3	51.604	18	.00004
mIdh-A	2	33.741	9	.00010
Ldh-A	2	16.628	9	.05487
Pep-B	3	103.158	18	.00000
Pgm-A	2	354.868	9	.00000
Pnp-A	4	394.455	27	.00000
sSod-A	2	30.491	9	.00036
Tpi-A	2	5.514	9	.78739
(Totals)		2097.070	315	.00000

Table 4. Summary of *F*-statistics calculated from 14 polymorphic loci among populations of *Lacerta nairensis*.

Locus	F_{IS}	F_{IT}	F_{ST}
sAat-A	1.000	1.000	.070
sAcoh-A	.619	.660	.106
Acp-B	.524	.838	.660
Cat-A	.373	.394	.033
Est-D	.358	.432	.115
βGlus-A	−.045	−.007	.036
βGa-1	1.000	1.000	.625
sIdh-A	.619	.660	.106
mIdh-A	−.181	−.032	.126
Pep-B	−.015	−.003	.013
Pgm-A	.273	.949	.930
Pnp-A	.383	.428	.074
sSod-A	1.000	1.000	.070
Tpi-A	−.017	−.003	.014
Mean	.503	.793	.584

Contingency Chi-square tests showed that 12 loci had significant ($P < 0.05$) heterogeneity in allele frequency, all populations considered together (table 3). The percentage of loci polymorphic (95% criterion) ranged from 0.0 to 16.67 for populations of *L. nairensis*, and between 8.33 and 11.1 for the populations of *L. raddei* (table 2). The mean number of alleles per locus, as well as mean heterozygosities, were generally higher

Table 5. Summary of F-statistics calculated from 15 polymorphic loci among populations of *Lacerta raddei*.

Locus	F_{IS}	F_{IT}	F_{ST}
sAat-A	−.017	−.004	.013
sAcoh-A	.213	.426	.270
mAcoh-A	−.017	−.004	.013
Acp-B	−.017	−.004	.013
Cat-A	−.017	−.008	.008
Ck-A	−.017	−.008	.008
Ck-C	−.017	−.004	.013
Est-D	.551	.588	.082
Gcdh-A	1.000	1.000	.038
βGlus-A	.233	.871	.832
Gsr-1	−.017	−.009	.009
sIdh-A	−.017	−.004	.013
Ldh-A	.649	.662	.038
Pep-B	.403	.480	.129
Pnp-A	.464	.956	.919
Mean	.380	.668	.465

Table 6. Summary of F-statistics calculated from 20 polymorphic loci among all populations of *Lacerta nairensis* and *Lacerta raddei*.

Locus	F_{IS}	F_{IT}	F_{ST}
sAat-A	.820	.832	.064
sAcoh-A	.296	.505	.297
mAcoh-A	−.017	−.002	.015
Acp-B	.485	.825	.660
Cat-A	.259	.279	.027
Ck-A	−.017	−.003	.013
Ck-C	−.017	−.002	.015
Est-D	.515	.584	.143
Gcdh-A	1.000	1.000	.045
βGlus-A	.179	.899	.877
Gsr-1	−.017	−.003	.014
βGa-1	1.000	1.000	.643
sIdh-A	.536	.583	.101
mIdh-A	−.181	−.019	.137
Ldh-A	.649	.665	.045
Pep-B	.393	.524	.216
Pgm-A	.273	.958	.942
Pnp-A	.405	.870	.781
sSod-A	1.000	1.000	.076
Tpi-A	−.017	−.002	.015
Mean	.430	.767	.591

for the *L. raddei* populations compared to the *L. nairensis* populations (table 2). The values of Wright's F-statistics were higher for the *L. nairensis* populations (tables 4, 5); there was relatively high heterozygote deficiency both within ($F_{IS} = 0.43$) and among ($F_{IT} = 0.767$) all the populations examined (table 6).

Table 7. Matrix of genetic distance coefficients among populations of *Lacerta nairensis* (1 = Yerevan, 2 = Aragatz, 3 = Adis 1, 4 = Adis 2, 5 = Tumanyan, 6 = Apnaguch) and *Lacerta raddei* (7 = Gosh, 8 = Chosrov, 9 = Geghart, 10 = Yekhegnadzor). Below diagonal: Nei's (1978) unbiased genetic distance. Above diagonal: Rogers' (1972) genetic distance.

	1	2	3	4	5	6	7	8	9	10
1	-	.027	.006	.021	.028	.042	.013	.046	.040	.061
2	.013	-	.029	.045	.055	.067	.032	.061	.058	.075
3	.006	.013	-	.024	.034	.048	.016	.049	.044	.065
4	.013	.027	.013	-	.049	.062	.030	.064	.059	.080
5	.023	.039	.026	.039	-	.017	.041	.074	.063	.089
6	.027	.044	.030	.043	.001	-	.046	.085	.078	.096
7	.001	.013	.001	.014	.027	.030	-	.048	.046	.058
8	.031	.042	.032	.046	.059	.063	.032	-	.061	.029
9	.029	.042	.029	.043	.049	.057	.030	.049	-	.079
10	.038	.048	.039	.053	.066	.067	.034	.005	.056	-

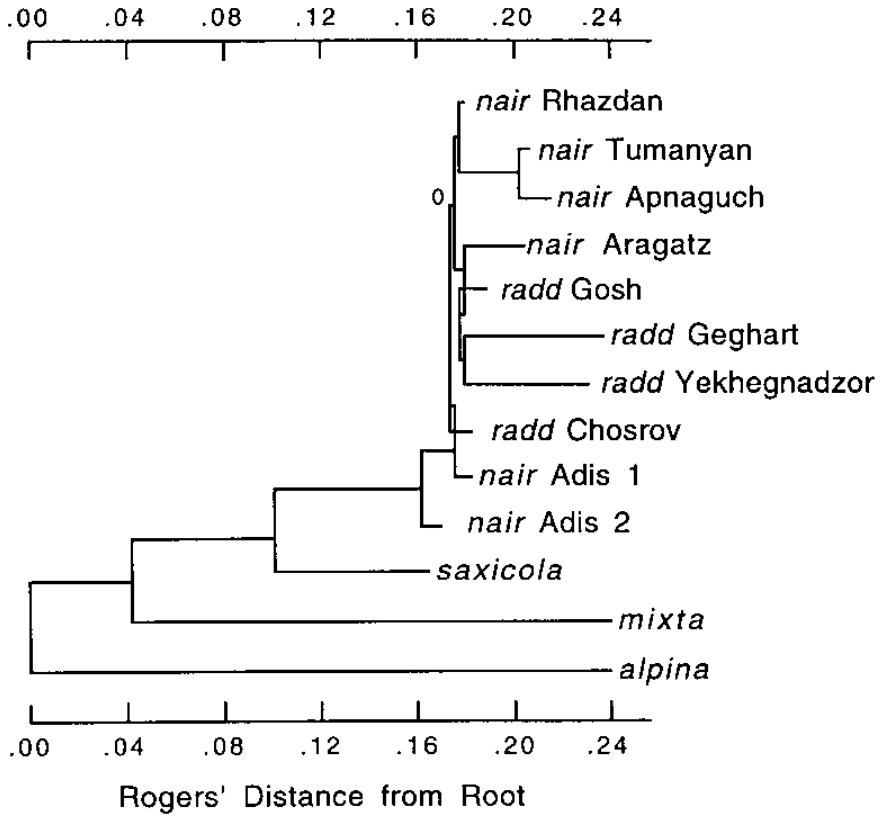

Figure 2. Distance Wagner tree (after optimization) produced by rooting with outgroup species. Goodness of fit statistics: percent standard deviation = 7.225; cophenetic correlation = 0.998.

Nei's (1978) genetic distance ranged from 0.001 to 0.044 among the six populations of *L. nairensis*, and from 0.005 to 0.056 among the four populations of *L. raddei* (table 7). Rogers' (1972) distances ranged from 0.006 to 0.067 among *L. nairensis* populations, and from 0.029 to 0.079 among *L. raddei* populations (table 7). The smallest distances were

between the Yerevan population of *L. nairensis* and the Gosh population of *L. raddei*, and between the Tumanyan and Apnaguch populations of *L. nairensis*; the greatest distances were between the Apnaguch population of *L. nairensis* and the Yekhegnadzor population of *L. raddei*.

In a Distance Wagner phenogram based on Rogers' (1972) genetic distances after optimization, the population of *L. raddei* from Gosh clustered with the six populations of *L. nairensis* rather than with the other populations of *L. raddei*. Another Distance Wagner phenogram rooted with three outgroup taxa (*L. saxicola, L. mixta,* and *L. alpina*), showed that the *L. raddei* populations clustered within the group of *L. nairensis* populations (fig. 2).

Discussion

The populations of *Lacerta raddei* and *L. nairensis* examined were monoallelic for almost half of the resolved loci; for several other loci, only one population showed any variation, usually when single individuals showed a unique allele. Measures of genetic variation within the populations were similar to those from Armenian populations of *L. valentini* and *L. portschinskii,* but generally lower than those for populations of *L. caucasica, L. daghestanica,* and *L. rudis* (Fu et al., 1995; MacCulloch et al., 1995). The percentages of polymorphic loci, including all loci with any variation (table 2), were generally lower than those reported for mainland populations of another lacertid, *Podarcis sicula* (27-45%; Gorman et al., 1975), and for teiids (*Cnemidophorus tigris, C. inornatus,* and *C. sexlineatus*: 15-34%; Gorman et al., 1977; Dessauer and Cole, 1984), and also lower than the average (21.7%) for other lizard species (summarized in Sattler and Ries, 1995).

Similarly, our measures of heterozygosity (table 2) were much lower than those reported for *Podarcis* (0.0588-0.1285; Gorman et al., 1975) and bisexual *Cnemidophorus* (0.04-0.1464; Gorman et al., 1977; Dessauer and Cole, 1984), as well the average value of 0.051 for several lizard species (Sattler and Ries, 1995). The low values of heterozygosity within populations of *L. raddei* and *L. nairensis* are more similar to values reported for fossorial or territorial, sit-and-wait lizards (Gorman et al., 1977), even though *Lacerta* are highly vagile and are active predators (Darevsky, 1967).

Reduced heterozygosity is also found in insular populations of lizards, which may be due to either founder effects, population bottlenecks, or small effective population sizes (Gorman et al., 1975). However, population sizes for many of the lizards sampled in our study were very high (unpubl. data) and sample sizes for almost all the populations were adequate to estimate mean heterozygosities. We also resolved enough loci in our study to estimate heterozygosity even in those populations with small sample sizes (Gorman and Renzi, 1979).

The low values of heterozygosity found within the populations we examined may reflect the numerous mountain populations of *Lacerta raddei* and *L. nairensis* (Darevsky, 1967), and suggest that each taxon is comprised of several disjunct populations through-

out its range despite a seemingly contiguous distribution (Darevsky, 1967; Moritz et al., 1992). In addition, the F_{ST} values for both taxa (tables 4, 5) are high enough to suggest a relatively high level of substructuring among populations, with reduced gene flow among them. However, the low overall genetic variation suggests that the various populations have not been separated long enough for the fixation of unique alleles. This is consistent with Darevsky's (1967) hypothesis of recent, post-glacial dispersals of *L. raddei* and *L. nairensis* into the Caucasus Mountains. There is also a trend towards higher heterozygosity values for the *L. raddei* populations, which may reflect the wider distribution of this taxon across the region compared to *L. nairensis* (Moritz et al., 1992). Future fieldwork should investigate the levels of dispersal among these populations, the dispersal abilities of these lizards, and any geographic restrictions to dispersal among populations.

If there is a correlation between lifestyle and heterozygosity within lizard genera as there is within families (Gorman et al., 1977), the slightly higher heterozygosity in *L. raddei* may also be related to its less territorial and aggressive nature compared to *L. nairensis* (Darevsky, 1967). However, the high standard errors associated with measures of genetic variation (table 2) render any such conclusions premature.

Our results support the work of Moritz et al. (1992) in that the population of *L. raddei* from Gosh clustered with the six populations of *L. nairensis* rather than with the other populations of *L. raddei*. Moritz et al. (1992) found that the mitochondrial DNA of *L. raddei* sampled from Gosh could not be distinguished from that of *L. nairensis*, except for a 200 base pair insertion; furthermore, mitochondrial DNA from the Gosh *L. raddei* was quite different than that sampled from two other populations of *L. raddei*. In our study, the genetic variation within the Gosh population of *L. raddei* was relatively high, with several alleles being present in this population and no other. The Gosh population of *L. raddei*, at the northeastern tip of Lake Sevan, is separated from other populations of *L. raddei* further to the north, east and south (see map in Moritz et al., 1992), and is far removed from the main range of this species to the southeast. It may have been isolated long enough for new alleles to enter the population, either through random drift or hybridization events with other species. The mitochondrial DNA evidence from Moritz et al. (1992) also suggests a close relationship between *L. raddei* from Gosh and at least two populations of *L. nairensis*.

There were no fixed allelic differences between *L. raddei* and *L. nairensis* in our study. Uzzell and Darevsky (1973) also could not distinguish the two species based on electrophoretic data from five loci. However, *L. raddei* and *L. nairensis* can be separated by differences in morphology and behavior, even where they are sympatric. *Lacerta raddei* has a white belly tinged with green, and has dark dorsal patterns that are restricted to the midline of the back. In contrast, *L. nairensis* has a yellow-green belly and dorsal markings that are scattered along the back. There are also minor scalation differences (Darevsky, 1967). *Lacerta nairensis* is generally larger in size than *L. raddei*, and is usually found at a higher elevation in a hill-steppe environment, rather than in the forest or foothills. The timing of oviposition and hatching occurs approximately one

month later in *L. nairensis*, but this may be due to the difference in altitude. During courtship and copulation, males of *L. nairensis* hold females at the thigh, a behavior shared by *L. raddei*, but *L. raddei* is unique in also grasping females on the side of the body (Darevsky, 1967). Although neither species seems to be very territorial, aggressive encounters between males are usually longer and more intense in *L. nairensis* compared to *L. raddei* (Darevsky, 1967).

The Distance Wagner phenogram, rooted with outgroup taxa (fig. 2), suggests that *L. raddei* and *L. nairensis* may not be monophyletic taxa. However, any phylogenetic interpretation of our distance-based tree is highly problematic for several reasons. First, several nodes in the tree have negative branch lengths, which has no phylogenetic meaning. Second, by their very nature, gene frequency data are predictably highly homoplastic. If two non-sister populations independently evolve gene frequencies for alternative alleles, then there is a 50% chance that the changes will be homoplastic unless selection is involved. If three populations evolve independently, then homoplasy must occur as at least two must evolve in a parallel direction, both either increasing or decreasing. Third, any phylogenetic interpretation of gene frequency data is suspect because gene frequencies are not heritable, but result from forces such as selection, drift, inbreeding, immigration and emigration (Murphy, 1993). Only heritable characters should be used in the reconstruction of genealogical relationships. Thus, we would not want to base a phylogeny on such highly homoplastic, non-heritable data. Consequently, because there is significant substructuring among the populations, but no fixed differences separating *L. raddei* and *L. nairensis*, we believe that our allozyme frequency data cannot be used to address the taxonomic status of the species. We are also uncertain which taxon is the maternal parent of *L. unisexualis*.

The discrepancy between the color, behavioral, and ecological data relative to the mitochondrial DNA restriction fragment length polymorphism data of Moritz et al. (1992) needs additional investigation before making a final decision on the taxonomic status of *Lacerta raddei* and *L. nairensis*. However, before making such a decision, additional fine-grained data, such as rapidly evolving mitochondrial DNA sequences, should be evaluated in the interest of maintaining nomenclatural stability.

Acknowledgements. We are grateful to J. Fu, G. Gollmann, H. Kaiser and an anonymous reviewer for their valuable comments on this manuscript. Import permits for frozen tissues were received from Agriculture Canada. 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. Kupriyanova. Laboratory work was carried out in the Laboratory of Molecular Systematics of the ROM, founded by a generous grant from NSERC.

For assistance with field work and providing specimens, we are extremely grateful to T. Morales-Sokolova, E. Roitberg, E. Yauruyan, M. Bakradze, V. Negmedzanov, D. Tarkhnishvili, I. Serbinova, B. Tuniyev, and especially N. Orlov and A. Agasian. A. Lathrop provided invaluable advice in the preparation of the figures.

Air France and KLM Airlines significantly contributed to our efforts by providing free excess baggage during international travels. This is contribution 13 from the Centre for Biodiversity and Conservation Biology, ROM.

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Appendix. Specimens collected.

Lacerta nairensis (n = 138). — Armenia: Yerevan, Rhazdan River, 40°11′50″N, 44°29′48″E, n = 47 (ROM 23754-23800); Gegam Range, Adis Mountain, 40°23′N, 44°42′E, n = 9 (ROM 23801-23803, 23805-23810); Sevan, 40°30′58″N, 44°56′26″E, n = 1 (ROM 23804); Aragatz Mountain, Amberd River, Bjurakan, 40°21′54″N, 44°15′12″E, n = 50 (ROM 23811-23860); Tumanyan, 41°00′00″N, 44°40′12″E, n = 19 (ROM 24780-24798); Apnaguch, 40°27′N, 44°22′E, n = 12 (ROM 24842-24843).

Lacerta raddei (n = 108). — Armenia: Yekhegnadzor, 39°45′N, 45°08′E, n = 3 (ROM 23619-23621); Geghart, 40°08′15″N, 44°49′06″E, n = 56 (ROM 23622-23677); Chosrov National Park, 40°00′54″N, 44°54′36″E, n = 40 (ROM 23678-23717); Gosh, 40°44′51″N, 45°01′26″E, n = 9 (ROM 23718-23726).

Received: October 16, 1995. Accepted: March 8, 1996.