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GENETIC AND MORPHOLOGICAL DIFFERENTIATION AMONG CAUCASIAN ROCK LIZARDS OF THE *Lacerta caucasica* COMPLEX

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The genetic diversity of 34 loci was surveyed from among five populations of the supposed conspecific taxa *Lacerta c. caucasica*, *L. c. alpina*, and *L. c. daghestanica*. Twelve loci exhibited variation. Fixed or nearly fixed allelic differences between *L. c. caucasica* and *L. c. daghestanica* were found at two loci, mannose-6-phosphate isomerase-A and creatine kinase-C. These two taxa differed from *L. c. alpina* at eight loci. Nei's (1978) genetic distance values among populations of *L. c. daghestanica* ranged from 0.000 – 0.029, between *L. c. caucasica* and *L. c. daghestanica* from 0.076 – 0.087 and between *L. c. alpina* and the other taxa from 0.472 – 0.501. Fixed allelic differences and consistent morphological character states support the recognition of these three taxa as separate species. Therefore, we recommend use of the names *L. alpina*, *L. caucasica*, and *L. daghestanica* for these taxa.

Key words: Reptilia, Lacertidae, *Lacerta*, genetics, systematics

INTRODUCTION

The subgenus *Archaeolacerta* has been the subject of much systematic investigation, mostly because of the extensive hybridization among species (e.g., Darevsky and Danielyan 1968; Darevsky et al. 1978, 1985). Among the many species of the Caucasus Mountains, *Lacerta caucasica* Méhely, 1909, has been considered a distinct, divergent member of the subgenus. It occurs in montane habitats throughout the Main Caucasian Range and in submontane areas north of the range. Evidence is provided that *Lacerta caucasica* can hybidize with *Lacerta saxicola* in some area in the Caucasus (Darevsky 1967; Darevsky et al. 1985).

Three subspecies are currently recognized: *L. caucasica caucasica*, *L. c. alpina*, and *L. c. daghestanica* (Darevsky 1984). Darevsky (1967) hypothesized that *L. c. caucasica* evolved from its ancestor *L. c.* (*saxicola*) *daghestanica* by montane isolation following the most recent glacial period. The latter taxon was placed as a subspecies of *L. caucasica* by Darevsky (1984). *Lacerta c. caucasica* and *L. c. daghestanica* were shown to exist sympatrically without morphological intergradation, at least at some localities. Roytberg and Lotiev (1992) and Roytberg (1994) suggested that species status may be warranted pending examination of additional specimens and from a greater geographic distribution.

Darevsky (1967, 1984) used morphological characters to distinguish *L. c. caucasica* from *L. c.* (*saxicola*) *daghestanica*. The former usually has a moderate midtemporal (masseteric) scale with 1 or 2 temporal (usually enlarged) scales between the masseteric and timpanic; number of femoral pores (Pfm) ranges from 9 to 19 ($\bar{X} = 43.5$), maximal shout-vent length 61 and 64 mm in males and females, respectively. In the latter taxon the midtemporal scale is tiny or absent, with more than 2 temporal scales between masseteric and timpanic; Pfm 13 – 18 ($\bar{X} = 15.7$), Sq 43 – 54 ($\bar{X} = 47.5$), maximal shout-vent length 54 and 58 mm in males and females, respectively.

Roytberg (1994) found a very clear separation between sympatric populations of the two taxa in multivariate analyses for several meristic characters, with Pfm and Sq contributing substantially into the discrimination.

Recognition of these taxa as species is dependent on demonstration of the cessation of gene flow, especially in the zone of sympatry between *L. c. caucasica* and *L. c. daghestanica*. Allozyme data may be highly applicable to solving this question of gene flow, and thus the taxonomic status of the subspecies.

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Not only are allozyme data independent of morphology, they also allow for direct estimations of gene flow. Often there is little correspondence between morphological and molecular data as they pertain to taxonomic status of a species (review in Murphy et al., in press). In order to provide an independent assessment of the taxonomic status of *L. c. caucasica* and *L. c. daghestanica*, and of the third subspecies, *L. c. alpina*, we undertook a molecular allozyme evaluation. We examined allozyme differentiation in three populations of *L. c. daghestanica*, one of *L. c. caucasica* and one of *L. c. alpina* to estimate gene flow among populations of a single taxon in this mountainous, geographically variable area. To address the taxonomic issue, our null hypothesis is that all taxa form a single species, i.e., that they form a single breeding unit with no restrictions to gene flow. Recognition of any or all taxa as species requires rejection of the null hypothesis.

MATERIALS AND METHODS

A total of 128 specimens of *L. caucasica* were collected from three localities in the Caucasian region of Daghestan during the summer of 1993. Specimens were collected in a submontane location at Gengutai (42° 60′ 36″ N, 47° 14′ 07″ E, elevation 771 m), at Kuli (42° 01′ 18″ N, 47°14′42″ E, elevation 1910 m), and Khvarshi (42°21′ N, 046°06′ E, elevation 1800 – 2200 m). Only *L. c. daghestanica* were collected at Gengutai ($N = 48$) and Kuli ($N = 49$); at the third location, Khvarshi, both *L. c. caucasica* ($N = 11$) and *L. c. daghestanica* ($N = 15$) were collected. Five specimens of *alpina* were collected on Aishkho Mountain near Krasnodar, Russia (45°02′ N, 39° 00′ E). Specimens were euthanized by an overdose of sodium pentobarbital. Tissues (heart, liver, skeletal muscle) were removed from most specimens immediately after euthanasia and frozen in liquid nitrogen. Some specimens were frozen whole in liquid nitrogen and dissected later. All voucher specimens are deposited in the collection of the Royal Ontario Museum (ROM; Appendix 1).

Enzymes were separated by horizontal starch gel electrophoresis on 11% gels. Homogenates of a combination of heart, liver and muscle tissues were used. The buffer systems used were Amine-citrate morpholine pH 6.1 and 7.5, Tris-citrate pH 7.0 and 8.0, Tris-citrate/borate pH 8.7, Tris-HCl pH 8.5, and Tris-borate EDTA, pH 8.6 (names from Murphy et al., in press). All procedures and protocols and enzyme and allelic nomenclature followed Murphy et al. (in press). The analysis utilized 27 enzyme systems encoded by 34 presumptive loci. Wherever possible, loci were resolved on two buffer systems to reveal hidden variability. Enzyme system names, Enzyme Commission numbers and electrophoretic buffer systems used for the loci are listed in Table 1. Electrophoresis was carried out on 2 specimens of *L. c. alpina* collected at Aishkho Mountain, 11 *L. c. caucasica* and 15 *L. c. daghestanica* from Khvarshi, 30 *L. c. daghestanica* from Kuli, and 32 *L. c. daghestanica* from Verkhnii Gengutai.

TABLE 1. Names and Enzyme Commission Numbers of Enzyme Systems Analyzed and the Buffer Systems Used in Analysis of 34 Loci in *Lacerta caucasica*. Names and Numbers Follow Those Used by Murphy et al. (in press)

Enzyme name and number	Buffer*
N-Acetyl-a-glucosaminidase (aGA) (EC 3.2.1.30)	2, 6
Acid phosphatase (ACP) (EC 3.1.3.2)	1
Aconitase hydratase (ACOH) (EC 4.2.1.3)	3, 4
Adenosine deaminase (ADA) (EC 3.5.4.4)	6
Aspartate aminotransferase (AAT) (EC 2.6.1.1)	1, 2
Calcium-binding proteins (CBP) (Nonspecific)	2, 6
Creatine kinase (CK) (EC 2.7.3.2)	4, 5
"Esterase-D" (Est-D) (EC 3.1.1.-)	6, 7
Glucose dehydrogenase (GCDH) (EC 1.1.1.118)	4, 5
Glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49)	1
Glucose-6-phosphate isomerase (GPI) (EC 5.3.1.9)	4, 7
a-Glucosidase (aGLUS) (EC 3.2.1.21)	6
a-Glucuronidase (aGLUR) (EC 3.2.1.31)	6
Glutamate dehydrogenase (GTD) (EC 1.4.1.2)	6
Guanine deaminase (GDA) (EC 3.5.4.3)	1, 6
Isocitrate dehydrogenase (IDH) (EC 1.1.1.42)	1, 2
L-Lactate dehydrogenase (LDH) (EC 1.1.1.27)	4, 6
Malate dehydrogenase (MDH) (EC 1.1.1.37)	1
Malate dehydrogenase ($NADP^+$) (MDHP) (EC 1.1.1.40)	4, 6
Mannose-6-phosphate isomerase (MPI) (EC 5.3.1.8)	2
Peptidase-A (Pep-A) (glycyl-leucine) (EC 3.4.-.-)	2, 3
Peptidase-B (Pep-B) (leucylglycylglycine) (EC 3.4.-.-)	2, 3
Phosphoglucomutase (PGM) (EC 5.4.2.2)	1, 2
Purine-nucleoside phosphorylase (PNP) (EC 2.4.2.1)	1, 2
Pyruvate kinase (PK) (EC 2.7.1.40)	3, 4
Superoxide dismutase (SOD) (EC 1.15.1.1)	5, 7
Triose-phosphate isomerase (TPI) (EC 5.3.1.1)	5

* 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-Cl, pH 8.2; 7) Tris-borate EDTA, pH 8.6.

TABLE 2. Genotype Frequencies for Polymorphic Loci in *Lacerta c. caucasica*, *L. c. daghestanica*, and *L. c. alpina*

Locus	*L. c. daghestanica* Gengutai	*L. c. daghestanica* Kuli	*L. c. daghestanica* Khvarshi	*L. c. caucasica* Khvarshi	*L. c. alpina* Mt. Aishkho
sAat-A	aa (32)	aa (28)	aa (15)	aa (10)	cc (2)
		ab (2)		ab (2)	
		ac (1)			
sAcoh-A	aa (24)	aa (30)	aa (10)	aa (11)	bb (2)
	ab (7)		ab (5)		
	bb (1)				
Acp-B	aa (31)	aa (20)	aa (14)	aa (11)	cc (2)
	ab (1)	ab (8)	ab (1)		
		bb (2)			
Ck-C	aa (32)	aa (30)	aa (15)	bb (10)	bb (2)
				ab (1)	
Gcdh-A	aa (31)	aa (30)	aa (15)	aa (11)	bb (2)
	ab (1)				
Gpi-A	aa (32)	aa (17)	aa (14)	aa (6)	aa (2)
		ab (11)	ab (1)	ab (4)	
		bb (2)		bb (1)	
Ldh-A	aa (32)	aa (27)	aa (15)	aa (11)	aa (2)
		ab (3)			
sMdhp-A	aa (32)	aa (30)	aa (10)	bb (6)	aa (2)
			ab (1)	ad (2)	
			ac (2)	dd (1)	
			ad (1)	de (1)	
				ee (1)	
Mpi-A	aa (32)	aa (30)	aa (15)	bb (11)	cc (2)
Pep-A	aa (32)	aa (19)	aa (15)	aa (9)	bb (1)
		ab (11)		(2)	
Pep-B	aa (30)	aa (21)	aa (13)	aa (11)	cc (1)
	ab (2)	ab (8)	ab (2)		cd (1)
		bb (1)			
Pgm-A	aa (32)	aa (30)	aa (14)	aa (11)	aa (2)
			ab (1)		
Pnp-A	aa (30)	aa (21)	aa (15)	aa (10)	aa (1)
	ab (2)	ab (7)		ab (1)	ab (1)
		ac (1)			
Tpi-A	aa (31)	aa (29)	aa (15)	aa (11)	cc (1)
	ab (1)	ab (1)			
PLP	2.70	18.92	5.41	5.41	5.41
MNA (± SE)	1.14 (0.06)	1.24 (0.09)	1.14 (0.06)	1.14 (0.06)	1.05 (0.04)
MHD (± SE)	0.011 (0.006)	0.049 (0.019)	0.018 (0.010)	0.022 (0.011)	0.027 (0.19)

PLP) Percentage of loci polymorphic (0.95 criterion), MNA) mean number of alleles per locus, MHD) mean heterozygosity by direct count.

Allozyme data were analyzed using BIOSYS-1 release 1.7 (Swofford and Selander 1989). All loci were evaluated for genetic polymorphism (percentage of loci exhibiting polymorphism, mean number of alleles per locus, mean heterozygosity), conformity to Hardy-Weinberg expectations using Levene's (1949) correction for small sample sizes, and for genetic structuring using Wright's (1978) *F*-statistics. Genetic divergence among populations was examined using genetic distance coefficients (Nei 1978; Rogers 1972). These were phenetically summarized by clustering using the Distance Wagner procedure (Farris 1972) of BIOSYS-1.

The specimens of *L. c. caucasica* and *L. c. daghestanica* were examined for morphological differences, using both Darevsky's (1967) and other criteria.

RESULTS

All taxa and populations were monoallelic at 22 of the 34 loci resolved including: mAat-A, mAcoh-A, Ada-A, Cbp-1, Ck-A, Est-D, Gda-A, aGlus-1, aGlur-1, Gpi-B, Gtdh-A, G6pdh-A, aGa-1, sIdh-A, mIdh-A, Ldh-B, sMdh-A, mMdh-A, mMdhp-A, Pk-A, sSod-A, and mSod-A. In most cases where more than one allele occurred at a locus, the less common alleles occurred in only one or two individuals. The exceptions to this were the six loci sAcoh-A, Acp-B, Gpi-A, Pep-A, Pep-B, and Pnp-A.

The distribution of locus polymorphism is summarized in Table 2. In addition to the 22 loci mentioned above, *L. c. caucasica* was homozygotic for six additional loci (sAcoh-A, Acp-B, Gcdh-A, Pep-B, Pgm-A, and Tpi-A) and *L. c. alpina* was homozygotic for nine additional loci (sAat-A, sAcoh-A, Acp-B, Ck-C, Gcdh-A, Gpi-A, Pep-A, Pgm-A, and Tpi-A). In *L. c. daghestanica* three loci (sAcoh-A, Acp-B, and Pep-B) exhibited greater heterozygosity than was observed in the other taxa. Only *L. c. daghestanica* at Gengutai exhibited variation at the locus Gcdh-A. At Kuli and Khvarshi, variation was found in both *L. c. caucasica* and *L. c. daghestanica* at 4 loci (sAat-A, Gpi-A, sMdhp-A, and Pep-A). These loci

TABLE 3. Contingency Chi-Square Analysis at All Variable Loci for Three Populations of *L. c. daghestanica*

Locus	No. of alleles	Chi-square	D. F	P
sAat-A	3	4.793	4	0.30916
sAcoh-A	2	103.957	2	0.00000
Acp-B	2	14.232	2	0.00081
Gcdh-A	2	0.493	2	0.78159
Gpi-A	3	28.104	4	0.00001
Ldh-A	2	4.793	2	0.09102
sMdhp-A	4	12.735	6	0.04744
Pep-A	2	18.559	2	0.00009
Pep-B	2	7.136	2	0.02821
Pgm-A	2	4.160	2	0.12491
Pnp-A	3	12.615	4	0.01332
Tpi-A	2	0.493	2	0.78159
Totals		212.071	34	0.00000

were not variable in the Gengutai population of *L. c. daghestanica.*

Only one locus failed to conform to Hardy – Weinberg expectations, sMdhp-A in *L. c. caucasica* (Chi-square = 19.335, D. F. = 6, $p = 0.004$). The Chi-square contingency tables are shown in Tables 3 – 4. Among the 12 loci that varied in *L. c. daghestanica*, seven showed significant frequency heterogeneity ($p < 0.05$; Table 3). When *L. c. caucasica* and *L. c. daghestanica* were combined (Table 4), nine loci exhibited significant heterogeneity.

The summary of *F*-statistics for all variable loci is shown in Table 5. The *F*-statistics showed an intrapopulation heterozygote deficiency ($F_{is} = 0.246$) for *L. c. caucasica* and a small heterozygote surplus in *L. c. daghestanica* ($F_{is} = -0.056$). There is a heterozygote surplus among the three populations of *L. c. daghestanica* ($F_{it} = 0.208$). An F_{st} of 0.250 indicates that these three populations do not form a panmictic group. Two loci exhibited fixed allelic differences between *L. c. caucasica* and *L. c. daghestanica*. The Gengutai and Kuli populations were invariant for the faster alleles at both Mpi-A and Ck-C. Because the lowland region around Gengutai is outside the range of *L. c. caucasica* (Darevsky 1967), these alleles are diagnostic for *L. c. daghestanica*. Specimens from Khvarshi, where both taxa are sympatric, can be unambiguously identified by alleles at these two loci. Fifteen of the 26 individuals from Khvarshi were homozygous for the faster alleles. The remaining 11 individuals all had slower alleles at Mpi-A and all but one had fixed slower alleles at the Ck-C locus;

TABLE 4. Contingency Chi-Square Analysis for All Variable Loci for *L. c. caucasica* and *L. c. daghestanica*

Locus	No. of alleles	Chi-square	D. F	P
sAat-A	3	5.618	6	0.46730
sAcoh-A	3	181.852	6	0.00000
Acp-B	2	18.238	3	0.00039
Ck-C	3	342.656	6	0.00000
Gcdh-A	3	3.693	6	0.71814
Gpi-A	2	23.131	3	0.00004
Ldh-A	2	5.901	3	0.11655
Ldh-B	2	176.000	3	0.00000
sMdhp-A	5	139.233	12	0.00000
Mpi-A	2	176.000	3	0.00000
Pep-A	2	18.101	3	0.00042
Pep-B	2	10.228	3	0.01673
Pgm-A	2	4.894	3	0.17969
Pnp-A	3	10.827	6	0.09389
Tpi-A	2	0.851	3	0.83715
Totals		1117.223	69	0.00000

TABLE 5. Summary of *F*-Statistics at All Variable Loci in *L. c. caucasica* and *L. c. daghestanica*

Locus	F_{is}	F_{it}	F_{st}
sAat-A	– 0.040	– 0.013	0.026
sAcoh-A	– 0.063	0.591	0.615
Acp-B	0.122	0.202	0.091
Gcdh-A	– 0.016	– 0.011	0.005
Gpi-A	– 0.105	0.050	0.140
Ldh-A	– 0.053	– 0.017	0.034
sMdhp-A	– 0.094	– 0.044	0.046
Pep-A	– 0.224	– 0.065	0.130
Pep-B	0.001	0.041	0.041
Pgm-A	– 0.034	– 0.011	0.022
Pnp-A	– 0.121	– 0.049	0.065
Tpi-A	– 0.016	– 0.011	0.005
Mean	– 0.056	0.208	0.250

a single individual was heterozygous at Ck-C. The slower alleles at these two loci are, therefore, diagnostic of *L. c. caucasica*. These two taxa are further distinguished by differences in allele frequency. In *L. c. caucasica*, four loci are monoallelic (sAcoh-A, Acp-B, Pep-B, and Tpi-A) whereas these are variable in *L. c. daghestanica.*

Greater genetic differentiation was observed between *L. c. alpina* and the two other taxa. Apparent fixed differences occurred at eight loci (mAcoh-A,

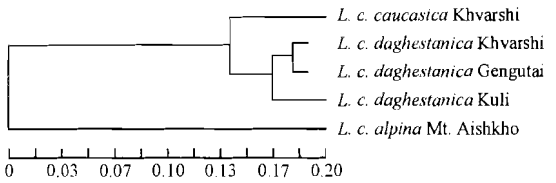

Fig. 1. Wagner tree produced by rooting at midpoint of longest path, based on Rogers' (1972) genetic distance (after optimization). Farris' (1972) "f" = 0.015; cophenetic correlation = 1.000. Total length of tree = 0.465.

Acp-B, Cbp-1, Gpi-B, Gtdh-A, Mpi-A, Pep-B, and Tpi-A). The slower Ck-C(b) allele was shared between *L. c. alpina* and *L. c. caucasica*. At Mpi-A, *L. c. alpina* possessed a unique allele thus not allowing for a hypothesis of allelic evolution at this locus.

Genetic distance coefficients (Nei 1978; Rogers 1972) among all taxa and populations of *L. c. daghestanica* are shown in Table 6. Figure 1 contains the Distance Wagner phenogram produced from the latter coefficients.

The morphological criteria specified by Darevsky (1967) to distinguish between *L. c. caucasica* and *L. c. daghestanica* produced a separation that differed from our allelic data. The specimens were therefore separated according to allelic states and reexamined for morphological characters that would allow them to be consistently distinguished. Specimens from all populations of *L. c. caucasica* and *L. c. daghestanica* were used in determining these characters. Three characters, including number of scales around midbody, number of scales along midline of throat and number of femoral pores, were found to consistently distinguish between *L. c. caucasica* and *L. c. daghestanica*. These morphological differences are summarized in Table 7.

TABLE 6. Genetic Distance Coefficients Among the Taxa and Populations of *Lacerta daghestanica*. Below Diagonal) Nei's (1978) Unbiased Genetic Distance; Above Diagonal) Rogers' (1972) Genetic Distance

No.	Taxon and locality	1	2	3	4	5
1	*L. c. caucasica* Khvarshi	****	0.089	0.098	0.090	0.387
2	*L. c. daghestanica* Gengutai	0.078	****	0.051	0.005	0.384
3	*L. c. daghestanica* Kuli	0.087	0.029	****	0.050	0.403
4	*L. c. daghestanica* Khvarshi	0.076	0.000	0.027	****	0.387
5	*L. c. alpina* Mt. Aishkho	0.472	0.473	0.501	0.475	****

DISCUSSION

The percentage of loci exhibiting polymorphism in the Gengutai and Khvarshi populations was approximately equivalent to the percentages in two Armenian populations of *Lacerta valentini* (PLP = 2.7 – 5.4), one of *L. portschinskii* (PLP = 5.56; unpublished data), and eight of *L. raddei* (including *L. nairensis*; PLP = 2.56 – 12.82; unpublished data). However, the population of *L. c. daghestanica* from Kuli had a much higher percentage of polymorphic loci, approaching that found in a population of *L. rudis* from Georgia (PLP = 25.0; unpublished data). None of these approached the PLP of 34% found in *Cnemidophorus tigris* by Gorman et al. (1977). The same pattern was seen in mean heterozygosity (MHD) and mean number of alleles per locus (MNA), with values from Khvarshi greater than those from Gengutai, *L. valentini* (MHD = 0.011 – 0.024, MNA = 1.08 – 1.16), *L. portschinskii* (MHD = 0.012, MNA = 1.08) and *L. raddei* (MHD = 0.009 – 0.027, MNA = 1.08 – 1.33) and those in the Kuli population much greater, although less than in *L. rudis* (MHD = 0.070, MNA = 1.42). Gorman et al. (1977) found heterozygosity of 0.0588 – 0.1285 in mainland populations of Podarcis sicula from the Adriatic coast, much greater than in any Caucasian species of lacertid examined herein. However, percentages of loci polymorphic in *P. sicula* were calculated without using the 0.95 criterion, as used in our study

TABLE 7. Some Morphological Characters Used to Differentiate Between *L. c. caucasica* and *L. c. daghestanica*. The Upper Line Contains the Range of the Number of Scales or Pores; the Lower Line Contains the Mean and Standard Deviation of these Numbers

Population	Body scales*	Throat scales**	Femoral pores
L. c. caucasica	7 – 45	17 – 20	12 – 15
Khvarshi (N = 11)	39.7 ± 2.2	19.1 ± 1.0	13.2 ± 1.1
L. c. daghestanica	45 – 57	20 – 28	15 – 18
Khvarshi (N = 15)	48.7 ± 2.9	22.3 ± 2.3	15.9 ± 0.9
L. c. daghestanica	46 – 58	21 – 27	14 – 19
Kuli (N = 49)	50.1 ± 2.7	24.2 ± 1.5	15.3 ± 1.1
L. c. daghestanica	40 – 52	16 – 24	13 – 18
Gengutai (N = 48)	46.3 ± 2.9	20.4 ± 1.7	14.5 ± 1.1

* Number of scales around midbody. Scales were counted at the position of the 15th transverse row of ventral scales. If ventral scale rows numbered more than 27 or fewer than 25, the position was shifted 1 or 2 rows backward or forward to maintain position at midbody.

** The number of scales along the midline of the throat to collar.

(Table 2). When PLP values are recalculated for our data without this criterion the values ranged from 17.65% to 26.47%, the highest value being again from Kuli. These values are still lower than those of *P. sicula*, which ranged from 27 – 45% (Gorman et al. 1975).

Heterozygosity values in *L. c. caucasica* and *L. c. daghestanica* in our study were lower than that reported in other active lizards (Lacertidae and Teiidae) and are more comparable to values of heterozygosity among "sit-and-wait" lizards (Gorman et al. 1977).

The range of genetic distances among populations of *L. c. daghestanica* was large. The populations at Kuli and Khvarshi exhibit smaller distance values from the population at Gengutai than they do from one another. Either the small sample sizes from Khvarshi or the high degree of polymorphism in the Kuli population may have been responsible for this result.

The single individual of *L. c. caucasica* heterozygous at Ck-C was placed in this taxon because it possessed the fixed, relatively slower Mpi-A allele, and was homozygous four other loci diagnostic of this taxon (sAcoh-A, Acp-B, Pep-B, and Tpi-A). However, it is possible that the specimen represents a backcross from a previous hybridization event. The faster Ck-C(a) allele is apparently fixed in *L. c. daghestanica*. Based on an outgroup analysis using the more distinctive *L. c. alpina* as the outgroup Ck-C(a) is also the derived allele. Thus the derived allele typical of *L. c. daghestanica* also occurs in *L. c. caucasica*, albeit relatively rarely. As an alternative explanation, it is possible that that the heterozygous condition was present in the common ancestor of *L. c. caucasica* and *L. c. daghestanica*, and thus the possession of both alleles is the plesiomorphic condition. However, this scenario seems less likely given the extreme rarity of heterozygotes in this enzyme system among reptiles (Buth et al. 1985). If the heterozygote is indicative of a hybridization event, such occurrences must be relatively rare and of little evolutionary consequence. Roytberg (1994) has reported specimens of apparent hybrids between *L. c. caucasica* and *L. c. daghestanica*, but only in narrow zones where the two taxa are sympatric. Our allozyme data at sMdhp-A supports a scenario of limited hybridization and restricted flow. In hybrid zones, we expect to the occurrence of novel alleles resulting from intercistronic recombination (e.g., Murphy et al. 1984, in press). Our data from sMdhp-A supports a conclusion of hybridization in the appearance of three alternative alleles not resolved in allopatric populations of the taxa, sMdhp-A(c), (d), and (e). The data are indicative of a narrow zone of integradation, a "hybrid sink, " and not one of unrestricted gene flow. Consequently, it appears as though the genetic integrity of the taxa is being maintained in sympatry, even given the likelihood of rare hybridization events.

It is clear from our analysis that we can diagnose these taxa and that hybridization has not resulted in significant introgression of alleles. The genetic integrity of the taxa has not been swamped out and it appears that such swamping will not occur. Therefore, we recommend elevation of the three subspecies to species status.

Elevation of *Lacerta alpina* Darevsky, 1967, to species status is warranted by the presence of fixed allelic differences at eight loci (Baverstock and Moritz 1990). The morphological characters specified by Darevsky (1967) will serve well as the description of the species.

Recognition of *Lacerta daghestanica* Darevsky, 1967, to species is warranted. Our data demonstrate that this taxon is not freely interbreeding with *L. caucasica*, even where they occur sympatrically. Thus, we reject our null hypothesis that these these taxa form a single gene pool. In part, this decision derives from the presence of apparent fixed allelic differences at two loci (Baverstock and Moritz 1990) and monomorphism in *L. caucasica* at four other loci that vary greatly in *L. daghestanica*. The two taxa can be distinguished by morphological characters (Table 5). Using the number of scales around midbody, number of scales along the midline of throat to collar and the number of femoral pores, *L. caucasica* Méhley, 1909, can be conclusively separated from the sympatric population of *L. daghestanica* at Khvarshi (see also Roytberg 1994), and from the other highland population at Kuli. However, there is overlap in these characters between *L. caucasica* and the population of *L. daghestanica* at Gengutai. Further molecular and morphological analyses using additional specimens may be helpful, especially from regions of allopatry of *L. caucasica*, and other regions of sympatry. The morphological divergence in regions of sympatry is noteable. In symparty we may be observing a classical example of character displacement, or in zones of allopatry character release.

The type locality of *L. caucasica* (Méhely 1909) is Mleti, Republic of Georgia. Although we have not

type locality falls outside the range of *L. daghestanica*. Consequently, although possible, we do not believe that *L. daghestanica* is referable to *L. caucasica* thereby making the former a junior synonym of the latter. However, if this is true, then the species currently referred to as *L. caucasica* would remain unnamed.

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APPENDIX

Specimens Examined. All specimens are deposited in the herpetological collections of the Royal Ontario Museum.

***Lacerta alpina*:** ROM 24371 – 24375, Russia, Krasnodar, Aishkho Mountain, 45° 02′ N, 39° 00′ E.

***Lacerta caucasica*:** ROM 24353 – 24357, 24359 – 24360, 24363, 24420, 24423, 24427, Russia, Daghestan, Khvarshi, 42° 21′ N, 46° 06′ E.

Lacerta daghestanica: ROM 23526 – 23559, Russia, Daghestan, Verkhnii Gengutai, 42° 40′ 36″ N, 47° 14′ 07″ E; ROM 23560 – 23607, Russia, Daghestan, Kuli, 42° 01′ 18″ N, 47° 14′ 42″ E; ROM 24358, 24361, 24362, 24413 – 24419, 24421 – 24422, 24424 – 24426, Russia, Daghestan, Khvarshi, 42° 21′ N, 46° 06′ E.

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