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BIOCHEMICAL, BEHAVIORAL AND BODY SIZE DIFFERENCES BETWEEN *RANA AURORA AURORA* AND *R. A. DRAYTONI*.—Red-legged frogs, *Rana aurora*, are secretive, aquatic to semi-aquatic anurans that range along the Pacific coast of North America from British Columbia to Baja California (Stebbins, 1966). Camp (1917) reduced the taxa, *R. draytoni* and *R. aurora*, to subspecific status based on a series of presumed intergrades from Mendocino County, California. He did not describe the attributes of the intergrades, and it is not clear why he made such an assignment. However, he defined *R. a. draytoni* by its larger size, rugose skin, prominent dorsolateral folds and distinct dorsal spots with light centers. We describe significant electrophoretic, behavioral and body size differences between representative populations of *R. a. aurora* and *R. a. draytoni*. Our original biochemical, ethological and morphological data are coupled with published observations. Using these data, we question further the current taxonomic designation of the two taxa.

Methods and materials.—Specimens of *R. a. aurora* (SVL = 25–38 mm; N = 20) and *R. a. draytoni* (SVL = 40–128 mm; N = 20) were collected in Del Norte and Santa Barbara counties, California, respectively. Voucher specimens (LACM 135300–319; 135325–344) were deposited in the Los Angeles County Natural History Museum.

Specimens were pithed within 24 hours of capture. Skeletal muscle, liver, and kidney tissues were removed and then stored at −78 C until used. Tissue extracts were prepared by mixing each tissue with an equal volume of 0.1 M Tris-HCl pH 7.0, mechanically homogenizing and centrifuging at 17,210 g at 4 C for 30 min. The supernatant fractions were collected and subjected to horizontal electrophoresis using 14% starch gels (80% Electrostarch; lot #392; Electrostarch Co., Madison, Wisconsin 53701, mixed with 20% Connaught starch; lot #374-1; Fisher Chemical Co., Ft. Lauderdale, Florida 33308). Table 1 lists the enzyme systems studied, and the buffer systems and electrophoretic conditions employed. Staining procedures followed Brewer (1970), Shaw and Prasad (1970), Selander et al. (1971) and Harris and Hopkinson (1977) with some minor modifications (Buth and Burr, 1978; Buth, 1979a; Buth and Murphy, 1980; Miyamoto, 1983b). General proteins were stained according to Buth (1979b). Creatine kinase was scored from gels stained for general proteins (Buth, 1982). Alleles of each presumptive locus were numbered according to increasing anodal mobility of their products.

Genetic differentiation between populations was calculated from allele frequencies using Nei (1972) coefficients of identity (I) and distance (D). Differences between genotypic frequencies of the two samples were tested with a G-test for independence ($\alpha = 0.05$; Sokal and Rohlf, 1981).

Reproductive behavior of *R. a. draytoni* was observed in single populations in San Luis Obispo and Santa Barbara counties, California. Standard lengths (snout-ischium lengths) were measured in the field to the nearest millimeter with a 15 cm plastic ruler. Maturity of measured frogs was determined by presence of nuptial pads in males, palpable enlarging ovaries in females, weight changes to detect enlarging ovaries in females of known age from tagged populations at the study sites, and examination of gonad size of dissected frogs. Comparable behavioral and morphological data for *R. a. aurora* were obtained from Storm (1960), Brown (1975) and Licht (1969, 1974), who based their studies on populations from Oregon, Washington, and British Columbia, respectively.

Results.—The gene products of 29 presumptive loci were consistently scored for all specimens of *R. a. aurora* and *R. a. draytoni*. A total of 41 allelic products was resolved (Table 2). Twenty loci (Ada-A, M-Aat-A, Ak-A, Cbp-1, Ck-A, Dlar, Pept-1, Est-1, Est-2, Gp-1, Gtdh-A, M-Icdh-A, S-Icdh-A, Ldh-A, Ldh-B, M-Mdh-A, S-Mdh-A, Pgm-A, S-Sod-A and Xdh-A) were monomorphically expressed between the samples. Of the nine polymorphic loci, one (S-Acon-A) was fixed for alternative alleles, whereas six others (M-Acon-A, S-Aat-A, Est-3, M-Me-A, S-Me-A and Mpi-A) exhibited significant differences in genotypic frequency (Table 3; $P < 0.05$). We observed no variation correlated with size in *R. a. draytoni* of different sizes.

Estimates of genetic variation for these taxa (heterozygosity, polymorphism, effective num-

TABLE 1. ENZYME SYSTEMS EXAMINED AND BUFFER SYSTEMS AND ELECTROPHORETIC CONDITIONS EMPLOYED. Enzyme nomenclature and enzyme commission numbers were taken from the International Union of Biochemistry, Nomenclature Committee (1979). Tissue extracts were either kidney (K), liver (L), or skeletal muscle (M). Buffer systems and electrophoretic conditions included: A) Sodium citrate pH 8.0 (Brewer, 1970) 8.5 V/cm for 7 hr; B) Tris-citrate pH 8.0 (Selander et al., 1971) 8.5 V/cm for 8 hr; C) Tris-hydrochloric acid pH 8.5 (Selander et al., 1971) 15.4 V/cm for 4 hr; D) Poulik pH 8.7 (Selander et al., 1971) 15.4 V/cm for 5.5 hr; E) Phosphate-citrate pH 7.0 (Selander et al., 1971) 5.4 V/cm for 12 hr; F) Tris-citrate-EDTA pH 7.0 (Avise et al., 1975) 10 V/cm for 8 hr.

Enzyme	Enzyme commission number	Locus	Tissue extract	Buffer system
Aconitate hydratase	4.2.1.3	M-Acon-A	L	A
		S-Acon-A	L	A
Adenosine deaminase	3.5.4.4	Ada-A	L	B
Adenylate kinase	2.7.4.3	Ak-A	M	B
Aspartate aminotransferase	2.6.1.1	M-Aat-A	M	C
		S-Aat-A	M	C
Calcium binding protein	—	Cbp-1	M	D
Creatine kinase	2.7.3.2	Ck-A	M	D
Dihydrolipoamide reductase	1.6.4.3	Dlar	L	E
Dipeptidase	3.4.13.11	Pept-1	M	C
Esterase	—	Est-1	L	D
		Est-2	K	D
		Est-3	K	D
General protein	—	Gp-1	L	D
Glucosephosphate isomerase	5.3.1.9	Gpi-A	L	D
Glutamate dehydrogenase	1.4.1.2	Gtdh-A	L	B
Isocitrate dehydrogenase	1.1.1.42	M-Icdh-A	L	E
		S-Icdh-A	L	E
Lactate dehydrogenase	1.1.1.27	Ldh-A	M	D
		Ldh-B	M	D
Malate dehydrogenase	1.1.1.37	M-Mdh-A	M	F
		S-Mdh-A	M	F
NADP-dependent malate dehydrogenase (="malic enzyme")	1.1.1.40	M-Me-A	M	F
		S-Me-A	M	F
Mannosephosphate isomerase	5.3.1.8	Mpi-A	M	D
Phosphoglucomutase	2.7.5.1	Pgm-A	M	F
Purine nucleoside phosphorylase	2.4.2.1	Pnp-A	L	B
Superoxide dismutase	1.15.1.1	S-Sod-A	L	E
Xanthine dehydrogenase	1.2.1.37	Xdh-A	K	D

ber of alleles) are shown in Table 4. Between the subspecies, values of 0.860 and 0.151 were obtained for the Nei genetic identity and distance coefficients, respectively.

Two significant differences in reproductive behavior were found between *R. a. aurora* and *R. a. draytoni*. All 31 male *R. a. draytoni* called from the water surface during the precopulatory phase of the breeding period. In contrast, males of *R. a. aurora* call from a submerged position during the precopulatory phase (Storm, 1960; Licht, 1969; Brown, 1975). Storm (1960) suggested that males of *R. a. aurora* also call above water, but we cannot determine from his data whether these calls were precopulatory advertisements.

The second behavioral difference is the location of egg masses. Twenty-four egg masses of *R. a. draytoni* were deposited on emergent vegetation such that the surface of the egg masses was at the water surface. In contrast, Storm (1960) and Licht (1969) suggested that *R. a. aurora* deposits eggs in the deepest water available such that the top surface of the egg mass is submerged from a few centimeters to many decimeters under water. Similarly, Brown (1975) reported submerged egg masses for this taxon.

TABLE 2. ALLELE FREQUENCIES AT NINE POLYMORPHIC LOCI BETWEEN TWO SAMPLES OF *R. a. aurora* AND *R. a. draytoni*. Twenty loci were monomorphically expressed between both taxa (Ada-A, M-Aat-A, Ak-A, Cbp-1, Ck-A, Dlar, Pept-1, Est-1, Est-2, Gp-1, Gtdh-A, M-Icdh-A, S-Icdh-A, Ldh-A, Ldh-B, M-Mdh-A, S-Mdh-A, Pgm-A, S-Sod-A, Xdh-A).

Locus	Allele	*R. a. aurora*	*R. a. draytoni*
M-Acon-A	1	1.00	0.30
	2	0.00	0.70
S-Acon-A	1	0.00	0.88
	2	1.00	0.00
	3	0.00	0.12
S-Aat-A	1	0.80	0.00
	2	0.20	1.00
Est-3	1	0.05	1.00
	2	0.95	0.00
Gpi-A	1	0.10	0.00
	2	0.90	1.00
M-Me-A	1	0.25	0.00
	2	0.75	1.00
S-Me-A	1	0.82	0.22
	2	0.18	0.78
Mpi-A	1	0.00	0.12
	2	0.00	0.05
	3	0.60	0.83
	4	0.40	0.00
Pnp-A	1	0.05	0.00
	2	0.95	1.00

TABLE 3. GENOTYPIC FREQUENCIES AT NINE POLYMORPHIC LOCI BETWEEN TWO POPULATION SAMPLES OF *R. a. aurora* AND *R. a. draytoni*. Significant differences (*G*-test; $P < 0.05$) between genotypic frequencies of the two population samples are indicated with an asterisk.

Locus	Genotype	*R. a. aurora*	*R. a. draytoni*
M-Acon-A*	1/1	20	0
	1/2	0	12
	2/2	0	8
S-Acon-A*	1/1	0	15
	1/3	0	5
	2/2	20	0
S-Aat-A*	1/1	14	0
	1/2	4	0
	2/2	2	20
Est-3*	1/1	1	20
	2/2	19	0
Gpi-A	1/1	1	0
	1/2	2	0
	2/2	17	20
M-Me-A*	1/1	2	0
	1/2	6	0
	2/2	12	20
S-Me-A*	1/1	14	0
	1/2	5	9
	2/2	1	11
Mpi-A*	1/2	0	2
	1/3	0	3
	3/3	7	15
	3/4	10	0
	4/4	3	0
Pnp-A	1/1	1	0
	2/2	19	20

Adult male and female *R. a. draytoni* (Table 5) are substantially larger (by 35–40 mm) than comparable adult *R. a. aurora* (Storm, 1960; Licht, 1974). Body size of the largest females of the Oregon population (Storm, 1960) barely overlaps the size of the smallest females of our California populations.

Discussion.—Case (1978a) included *R. aurora* in her analysis of western *Rana*, but we cannot compare our data with hers because she pooled several populations probably representing both taxa, and moreover, more than half the loci in our study were unique. Nevertheless, our Nei genetic distance coefficient ($D = 0.151$) is similar to values observed for closely related species of lizards (Buth et al., 1980), and this value falls in between values found between populations ($D = 0.01$–0.05) and non-sibling species ($D = 0.68$–0.77) for ranid frogs (Case, 1978b), and other vertebrates (Adest, 1977; Murphy and Ottley, 1980; Ferris et al., 1982).

We recognize the limitations associated with employing the Nei distance as an exclusive measure of taxonomic relatedness (Buth and Mayden, 1981). To uncover any differences the Nei value might mask, we analyzed statistically genotypic frequency differences at the nine polymorphic loci (Table 3). Significant differences were found at seven loci (S-Acon-A, M-Acon-A, S-Aat-A, Est-3, M-Me-A, S-Me-A, and Mpi-A), suggesting wide genetic differentiation between

TABLE 4. ESTIMATES OF GENETIC VARIATION WITHIN TWO POPULATION SAMPLES OF *R. a. aurora* AND *R. a. draytoni*. Loci were considered polymorphic if the most common allele of a population sample occurred at a frequency less than or equal to 0.95. All calculations were based on the allelic products of 29 presumptive loci.

Taxon	Proportion of loci heterozygous per individual	Proportion of loci polymorphic per population	Effective number of alleles per locus
R. a. aurora	0.053	0.241	1.098
R. a. draytoni	0.046	0.138	1.067

the two forms (Buth et al., 1980; Miyamoto, 1983a).

The behavioral differences we observed are similar to those observed between other ranid species (Zweifel, 1955; Licht, 1969). The adult size differences between *R. a. aurora* and *R. a. draytoni* (Table 6) is greater than that found between other species pairs of western ranid frogs (i.e., *R. boylei, R. cascadae, R. muscosa* and *R. pretiosa*) (Zweifel, 1955; Licht, 1969; Briggs and Storm, 1970).

Behavioral and morphological differences correspond to allozyme differences. The three data sets suggest significant differences between *R. a. aurora* and *R. a. draytoni*. Differences between these frog taxa pose problems similar to those found in certain well-differentiated populations of the fish, *Catostomus plebeius* (Ferris et al., 1982). We follow Buth and Mayden (1981) in our conviction that the geographic pattern of genetic differentiation rather than its absolute magnitude should be the primary criterion in evaluating the taxonomic status of these frogs.

As in *Catostomus*, our data do not distinguish between clinal and stepwise differentiation at the contact zone between the forms. Unfortunately, the existing literature is vague regarding the position and extent of the contact zone (Altig and Dumas, 1972). Camp (1917) defined this zone as the point location, Mendocino City, Mendocino County, California. Grinnell and Camp (1917) extended the zone to include western Mendocino and Humboldt counties, California. Storer (1925) stated that typical *R. a. draytoni* extends northward to Gualala, and 4.8 km west of the summit of Mount Sanhedrin, Mendocino County. Because the electrophoretic data set for *R. a. aurora* comes from a different geographic location than those from which the behavioral and morphological data sets were obtained, we cannot eliminate, however, unlikely, the possibility that the observed differences among the data sets conform to nonparallel clines. Further study in the contact zone between these taxa is needed to resolve the taxonomic status of these frogs.

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TABLE 5. SUMMARY OF ADULT STANDARD LENGTHS OF *R. a. aurora* AND *R. a. draytoni*.

Taxon	Sex	N	Range in standard length (mm)	Mean (mm)	Population	Source
R. a. aurora	♂♂	>69	45–60	—	Fraser River, BC	Licht (1974)
	♀♀	>123	62–80	—	Fraser River, BC	Licht (1974)
	♂♂	11	49–65	59	Corvallis, OR	Storm (1960)
	♀♀	9	72–93	84	Corvallis, OR	Storm (1960)
R. a. draytoni	♂♂	85	78–116	101	San Luis Obispo, CA	Present study
	♀♀	42	91–138	120	San Luis Obispo, CA	Present study
	♂♂	16	82–108	92	Santa Barbara, CA	Present study
	♀♀	17	87–129	112	Santa Barbara, CA	Present study

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