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SHEPPARD, AND J. D. WILLIAMS. 1985. Endangered and threatened wildlife and plants; review of vertebrate wildlife; notice of review. Part III. U.S. Department of Interior, Fish and Wildlife Service. Federal Register 50:37958-37967.

HERKERT, J. R. 1992. Endangered and Threatened Species of Illinois: Status and Distribution. Vol. 2—Animals. Illinois Endangered Species Protection Board, Springfield.

PAUKSTIS, G. L., AND L. E. BROWN. 1987. Evolution of the intercalary cartilage in chorus frogs, genus *Pseudacris* (Salientia: Hylidae). Brimleyana 13:55-61.

———, AND ———. 1991. Evolutionary trends in the morphology of the intercalary phalanx of anuran amphibians. Can. J. Zool. 69:1297-1301.

SAS INSTITUTE. 1988. SAS/STAT user's guide. SAS Institute, Cory, North Carolina.

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Nuclear Ribosomal DNA Restriction Sites, Phylogenetic Information, and the Phylogeny of Some Xenodontine (Colubridae) Snakes

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The use of sequence and restriction site data from the nuclear rDNA repeat region has become popular for a wide variety of phylogenetic studies at all classification levels (see reviews by Mindell and Honeycutt, 1990 and Hillis and Dixon, 1991). Despite this popularity, the nuclear rDNA repeat has never been examined in snakes (Sauria: Serpentes) for phylogenetic information and only a single study (Cortadas and Pavon, 1982) has looked at the repeat in snakes at any level. Cortadas and Pavon (1982) characterized the organization of the rDNA repeat in 15 species of vertebrates through restriction mapping with two endonucleases, *Eco*RI and *Bam*HI. Included in their study were three species of snakes, one viperid (*Bothrops neuwiedi*) and two colubrids (*Dromicus* = *Liophis poecilogyrus* and *Natrix maura*). Because of the paucity of information on the nuclear rDNA repeat in snakes, we decided to restriction map the rDNA repeat in several taxa of snakes within the phylogenetically enigmatic subfamily Xenodontinae. The purpose of the mapping was two-fold: (1) to characterize snake repeats in greater detail than done previously, and (2) to examine the utility of rDNA restriction site data for estimating phylogeny at the intra-subfamily level in snakes.

A total of 16 species of snakes from six genera within the Xenodontinae were included in the study (Appendix 1). Whole genomic DNA was isolated from frozen (−80 C) liver and/or muscle tissue. All protocols and assays for isolation, enzyme digestion, hybridization, autoradiography, and fragment size determination follow those of Davis (1986) and Hillis and Davis (1986). Two probes (both from *Geomys*) were used to identify rDNA fragments. One probe, pGb28S contained the region located between two conserved *Eco*RI sites which included part of the 18S, both ITS, the entire 5.8S and most of the 28S. The other probe, pGb18S, contained the region located between a conserved *Eco*RI and *Hind*III site which included most of the 18S and most of the ETS. Fourteen Type II restriction enzymes were employed in the study and these were *Apa*I, *Bam*HI, *Bcl*I, *Bgl*II, *Bste*II, *Dra*I, *Eco*RI, *Eco*RV, *Hinc*II, *Hind*III, *Kpn*I, *Pst*I, *Pvu*II, and *Xba*I. Restriction site maps were constructed from double digest information.

TABLE 1. Data matrix of the informative restriction sites, i.e., only those sites which have synapomorphic content. − = absent, + = present, ? = missing.

OTUs	Characters							
	1 Bste II	2 Bste II	3 Bste II	4 Dra I	5 Kpn I	6 Bam HI	7 Sac I	8 Bam HI
antillensis	?	+	−	−	+	+	−	?
callilaemum	−	−	+	−	−	+	−	?
vudii	?	+	−	−	−	−	+	+
polylepis	−	−	+	−	−	+	−	?
frenatus	?	+	−	−	−	+	−	?
abacura	?	+	−	−	−	−	−	−
cantherigerus	?	+	−	−	−	−	+	+
parvifrons	+	+	−	−	−	?	−	?
exiguum	+	+	−	−	−	−	−	+
ferox	?	+	−	−	−	+	−	?
funereum	−	−	−	−	−	+	−	?
rijersmai	−	−	−	−	+	−	−	+
portoricensis	?	+	−	−	−	+	−	?
elegans	?	?	−	+	−	−	−	+
oxyrhynchus	?	?	−	+	−	−	−	−
catesbyi	?	+	−	+	−	+	−	?

For the phylogenetic analyses, the restriction site data were coded as presence/absence characters. They were analyzed following the constraints of Dollo parsimony (Farris, 1977; Debry and Slade, 1985; Swofford and Olsen, 1990) because the probabilities of character change, i.e., gain of a site versus loss of a site, are asymmetrical (Templeton, 1983); the convergent loss of a site is more likely than the convergent gain of a site. Dollo parsimony handles this descrepancy in probability by constraining the evolution of all gains as unique in the phylogenetic analysis (Swofford and Olsen, 1990), which in effect acts as a weighting procedure, making site gains more informative than site losses.

Taxonomic outgroup designations were determined based on relationship estimates of these snakes from allozyme data, i.e., *Alsophis* species were employed as a paraphyletic outgroup (Crother, unpubl. data). However, we did not constrain the analyses to those outgroups but instead employed each taxon at least once as the root in the event that an unlikely

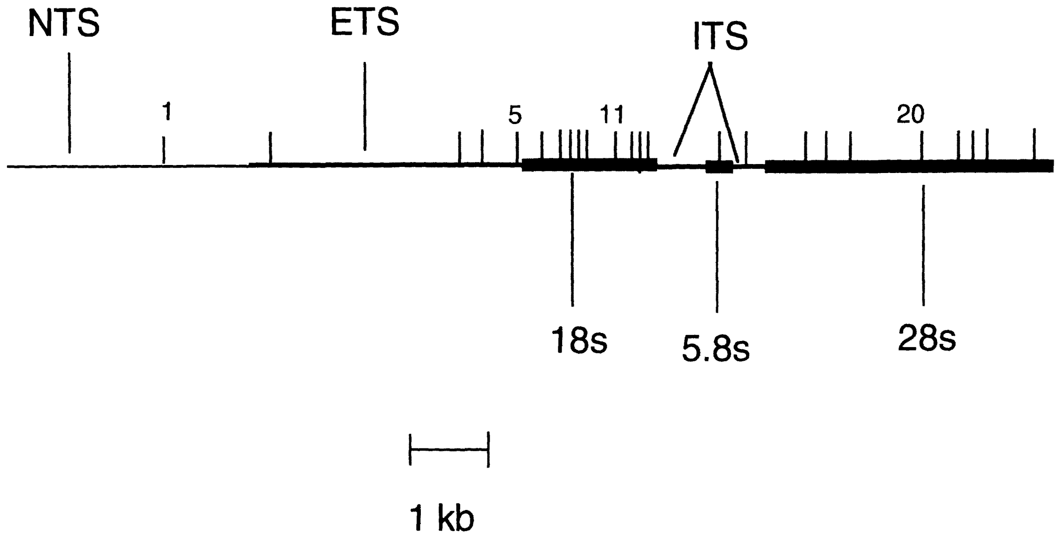

FIG. 1. Restriction endonuclease map of the sites conserved in all snake taxa surveyed. Each vertical line represents a restriction site. Key: 1—*Pst*I, 2—*Hind*III, 3—*Hinc*II, 4—*Hind*III, 5—*Dra*I, 6—*Xba*I, 7—*Bste*II, 8—*Bam*HI, 9—*Pst*I, 10—*Hinc*II, 11—*Bste*II, 12—*Eco*RI, 13—*Xba*I, 14—*Bcl*I, 15—*Kpn*I, 16—*Hinc*II, 17—*Bam*HI, 18—*Dra*I, 19—*Sac*I, 20—*Bam*HI, 21—*Pvu*II, 22—*Hinc*II, 23—*Sac*I, 24—*Eco*RI.

outgroup provided a more parsimonious estimate of phylogeny. All the phylogenetic analyses were conducted using PAUP (Phylogenetic Analysis Using Parsimony, version 3.0, Swofford, 1991). The Branch and Bound option was used to find the most parsimonious trees. The amount of phylogenetic signal in the data was examined by obtaining an estimate of the skewness of the tree length frequency distribution (Hillis, 1991; Huelsenbeck, 1991; Hillis and Huelsenbeck, 1992).

The restriction mapping yielded eight variable sites with phylogenetic information and these sites came from only five enzymes (Table 1). Twenty-four mapped sites were conserved in all the taxa surveyed (Fig. 1) and 25 mapped sites were interspecifically variable (Fig. 2). Repeat lengths varied over a broad range (16.8 kb-23.8 kb, Table 2). Some of the sites mapped as conserved displayed minor variation in location. Three of the 14 restriction enzymes yielded no usable data, but for different reasons. *Bgl*II did not cut for most taxa and could not be mapped unambiguously. *Eco*RV had no sites within the repeat and *Apa*I cut in an excessive number of sites (or consistently resulted in severe partial digests), yielding data not amenable to confident mapping of those sites.

The phylogenetic analysis of the restriction site data resulted in 4637 most parsimonious trees, each with a length of 12 steps, a consistency index (CI; Kluge and Farris, 1969) of 0.667 and a retention index (Farris, 1989) of 0.938. None of these descriptive statistics varied over any of the 16 outgroup analyses. Strict consensus and majority rule consensus trees (Fig. 3) indicate minimal consistent structure. However, what structure there is suggests (1) *Alsophis vudii* and *A. cantherigerus* are sister taxa, (2) the Jamaican radiation of *Arrhyton* is monophyletic, and (3) the two non-Antillean xenodontine taxa, *Farancia abacura* and *Alsophis elegans* may be internested within the Antillean assemblage. The random sampling of tree length frequency distributions yielded a (50,000 trees) $g_1 = -0.017$, which is not significantly different from a normal distribution (Hillis and Huelsenbeck, 1992).

The organization of the rDNA repeat into three coding regions, three transcribed spacers, and a non-transcribed spacer, which all evolve at different rates (Appels and Dvorak, 1982; Hillis and Davis, 1986), is

TABLE 2. Total length of a single copy of the rDNA repeat in the taxa surveyed. The repeat length is based on the fragment size yielded from *Bcl* I, which has only a single conserved site in the 5.8S gene of the repeat. Repeat lengths are in kilobases.

Alsophis vudii	16.8	*Alsophis cantherigerus*	19.6
Hypsirhynchus ferox	20.2	*Farancia abacura*	20.4
Arrhyton polylepis	20.6	*Antillophis parvifrons*	20.6
Alsophis antillensis	21.0	*Alsophis rijersmai*	21.1
Arrhyton callilaemum	21.8	*Uromacer oxyrhynchus*	21.9
Arrhyton funereum	22.3	*Alsophis elegans*	22.6
Uromacer frenatus	22.8	*Alsophis portoricensis*	23.0
Arrhyton exiguum	23.1	*Uromacer catesbyi*	23.8

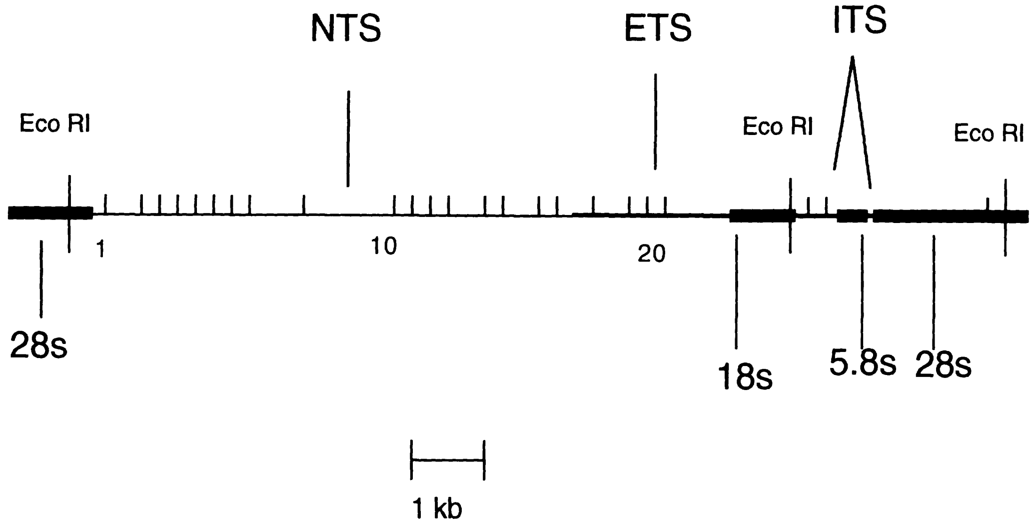

FIG. 2. Restriction endonuclease map of the sites that varied interspecifically, including autapomorphic sites. Each vertical line represents a site. The *Eco*RI sites are for reference. *Represents phylogenetically informative sites. Key: 1—*Xba*I, 2—*Dra*I, *3—*Bam*HI, 4—*Pvu*II, 5—*Kpn*I, *6—*Sac*I, *7—*Bam*HI, *8—*Bste*II, 9—*Kpn*I, *10—*Bste*II, 11—*Bam*HI, 12—*Eco*RI, 13—*Bcl*I, *14—*Kpn*I, 15—*Pvu*II, 16—*Sac*I, *17—*Bste*II, 18—*Kpn*II, 19—*Dra*I, 20—*Bam*HI, 21—*Bam*HI, 22—*Dra*I, 23—*Hind*III, *24—*Dra*I.

such that apriori one would expect phylogenetic information from the repeat at a variety of taxonomic levels of comparison. In this subset of snakes all within the same subfamily, there were nearly the same number of conserved sites and variable sites, and almost all of the variable sites were unique to single taxa.

The locations of the variable sites and conserved sites followed the expectations based on the known relative rates of change for each region. All but eight of the variable sites were located in the non-transcribed spacer and seven of the remaining variable sites were located in the transcribed spacers. A single variable site (*Dra*I) was located in the 28S region. All but six of the 24 conservative sites were located in the coding regions, and of the these six, only one was located in the non-transcribed spacer.

The absence of shared derived sites was a departure from the amount of phylogenetic information derived from nuclear rDNA restriction sites in other taxonomic groups of about the same taxonomic level. For example, rDNA restriction site studies on the genus *Rana* (Hillis and Davis, 1986), phyllostomatid bats (Van Den Bussche, 1991), and mammals in the subfamily Bovinae (Wall et al., 1992) all yielded substantial numbers of informative sites. In the present study, only eight informative sites were found. Why is there such a paucity of phylogenetically informative sites for this group of snakes? It has been suggested previously by Cadle (1988) that the molecular evolution of snakes is conservative relative to other groups. This is perhaps one reason, but we refrain from speculating on other possibilities.

What was resolved, however, is of interest. The phylogeny of xenodontine snakes remains quite unknown, despite the efforts of Cadle (1984a, b, c). Most of the snakes in this study are part of the Antillean assemblage, endemic to the islands in the Caribbean. Maglio (1970) presented the only phylogenetic hypothesis of this assemblage. Our results (based on the strict consensus tree) support his sister relationship between *Alsophis vudii* (Bahamas) and *A. cantherigerus* (Cuba), but our results disagree with Maglio's nonmonophyletic depiction of the Jamaican *Arrhyton.* Based on the taxa we examined, the prediction from Maglio's work would have been that *A. callilaemum* would be the sister to *A. exiguum.* We found the three Jamaican species *funereum, polylepis,* and *callilaemum* formed a monophyletic group (this is supported by unpublished allozyme data of BIC). The small number of informative sites precluded any other firm systematic conclusions, but hinted at the possibility that the Antillean assemblage of xenodontines is non-monophyletic due to the internesting of *Farancia* and mainland *Alsophis.*

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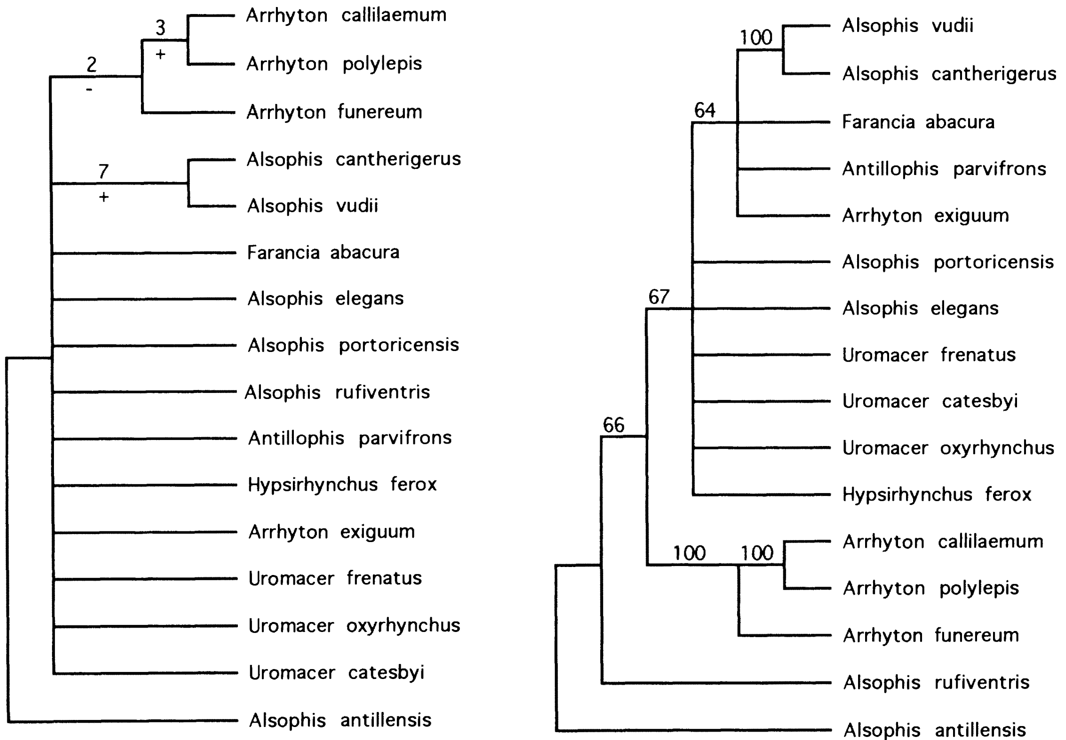

FIG. 3. A. Strict consensus tree. The numbers represent unambiguous characters and the symbols below represent the character state. B. 50% majority-rule tree. The numbers represent the percentage the clades were found in most parsimonious trees. Unlabelled nodes were present in less than 50% of the trees.

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LITERATURE CITED

APPELS, R., AND J. DVORAK. 1982. Relative rates of divergence of spacer and gene sequences within the rDNA region of species in the Triticeae: implications for the maintenance of homogeneity of a repeated gene family. Theor. Appl. Genet. 63: 361–365.

CADLE, J. E. 1984a. Molecular systematics of neotropical xenodontine snakes: I. South American xenodontines. Herpetologica 40:8–20.

———. 1984b. Molecular systematics of neotropical xenodontine snakes: II. Central American xenodontines. Herpetologica 40:21–30.

———. 1984c. Molecular systematics of neotropical xenodontine snakes: III. Overview of xenodontine phylogeny and the history of New World snakes. Copeia 1984:641–652.

———. 1988. Phylogenetic relationships among advanced snakes: a molecular perspective. Univ. California Publ. Zool. 119:1–77.

CORTADAS, J., AND M. C. PAVON. 1982. The organization of ribosomal genes in vertebrates. EMBO 1:1075–1080.

DAVIS, S. K. 1986. Population structure and patterns of speciation in *Geomys* (Rodentia: Geomyidae): an analysis using mitochondrial and ribosomal DNA. Unpubl. Ph.D. Diss., Washington Univ.

DEBRY, R. W., AND N. A. SLADE. 1985. Cladistic analysis of restriction endonuclease cleavage maps within a maximum-likelihood framework. Syst. Zool. 34:21–34.

FARRIS, J. S. 1977. Phylogenetic analysis under Dollo's law. Syst. Zool. 26:77–88.

———. 1989. The retention index and the rescaled consistency index. Cladistics 5:417–419.

HILLIS, D. M. 1991. Discriminating between phylogenetic signal and random noise in DNA sequences. *In* M. M. Miyamoto and J. Cracraft (eds.), Phylogenetic Analysis of DNA Sequences, pp. 278–294. Oxford Univ. Press, New York.

———, AND S. K. DAVIS. 1986. Evolution of ribosomal DNA: fifty million years of recorded history in the frog genus *Rana*. Evolution 40:1275–1288.

———, AND M. T. DIXON. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66:411–453.

———, AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. J. Heredity 83:189–195.

HUELSENBECK, J. P. 1991. Tree-length distribution skewness: an indicator of phylogenetic information. Syst. Zool. 40:257-270.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. 18:1-32.

MAGLIO, V. J. 1970. West Indian xenodontine colubrid snakes: their probable origin, phylogeny, and zoogeography. Bull. Mus. Comp. Zool. 141:1-54.

MINDELL, D. P., AND R. L. HONEYCUTT. 1990. Ribosomal RNA in vertebrates: evolution and phylogenetic applications. Annu. Rev. Ecol. Syst. 21: 541-566.

SWOFFORD, D. L. 1991. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.0. Illinois Natl. Hist. Surv., Champaign.

———, AND G. J. OLSEN. 1990. Phylogeny reconstruction. *In* D. M. Hillis and C. Moritz (eds.), Molecular Systematics, pp. 411-501. Sinauer Associates, Sunderland, Massachusetts.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes. Evolution 37:221-244.

VAN DEN BUSSCHE, R. A. 1991. Phylogenetic analysis of restriction site variation in the ribosomal DNA complex of New World leaf-nosed bat genera. Syst. Zool. 40:420-432.

WALL, D. A., S. K. DAVIS, AND B. M. READ. 1992. Phylogenetic relationships in the subfamily Bovinae (Mammalia: Artiodactyla) based on ribosomal DNA. J. Mamm. 73:262-275.

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APPENDIX I

Voucher Information for Specimens used in the Study

Alsophis antillensis: RLB15P58a; *Alsophis cantherigerus*: CUBA: Guantanamo U.S. Naval Base; Cuzco Beach, UMRC 86-1; *Alsophis elegans*: PERU: Lima; Paraiso, JEC 7146; *Alsophis portoricensis*: PUERTO RICO: Isla de Culebra, USC 8073; *Alsophis rijersmai*: ANGUILLA: MPM 23411; *Alsophis vudii*: BAHAMAS: Berry Islands; Frazer's Hog Cay, UMRC 86-38; *Antillophis parvifrons*: HAITI: l'Ouest; Croix des Bouquets, UMRC 86-27; *Arrhyton callilaemum*: JAMAICA: St. Andrews Parish; Maryland Town, USC 7599; *Arrhyton exiguum*: PUERTO RICO: Poblado Castaner, USC 8071; *Arrhyton funereum*: JAMAICA: Westmoreland Parish; Bethel Town, USC 7237; *Arrhyton polylepis*: JAMAICA: Portland Parish; Windsor Forest, USC 7869; *Farancia abacura*: USA: southern Florida; Tamiami Trail 10 mi. W Krome Ave., TNHC 50103; *Hypsirhynchus ferox*: HAITI; Ile de Gonave; Etroits, UMRC 86-34; *Uromacer catesbyi*: HAITI: l'Ouest; Croix des Bouquets, UMRC 86-17; *Uromacer frenatus*: HAITI: l'Ouest; Croix des Bouquets, UMRC 86-23; *Uromacer oxyrhynchus*: HAITI: l'Ouest; Croix des Bouquets, UMRC 86-15.

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A Simple Test of Prey Discrimination that Demonstrates Learning in Postlarval Ranid Frogs

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Effective methods for demonstrating learning in postlarval anurans are uncommon, especially for members of the family Ranidae. Ranids typically have a sedentary lifestyle and are consequently often unresponsive in conventional assays such as maze learning (see Suboski, 1992). Furthermore, active avoidance learning is difficult to demonstrate (McGill, 1960; Boice, 1970; Thompson and Boice, 1975), since many ranid species often respond to negative stimuli by remaining motionless rather than running away (Gregory, 1979; Licht, 1986). Therefore, any test of learning in postlarval ranids must consider their specialized behavioral patterns.

Many anuran species will readily strike at any appropriately shaped moving object, even if the item is non-living (Sternthal, 1974; Ewert, 1976; Ewert and Burghagen, 1979; Mikulka et al., 1980). Because of this non-selective response toward potential prey items, it might be adaptive for anurans to have the ability to rapidly learn to discriminate between palatable and unpalatable prey items. For example, bufonids will quickly learn to reject stinging insects and their mimics but continue to strike at other prey items (Brower et al., 1960; Brower and Brower, 1962). Visual characteristics such as size, shape, and/or color are probably the primary cues that allow anurans to differentiate between prey items (Ewert, 1976; Ewert and Burghagen, 1979; Mikulka et al, 1980). However, *Rana pipiens* can use olfactory cues to discriminate between visually identical prey items (Sternthal, 1974).

Examination of prey-discriminating abilities provides an appropriate assay for testing the learning and memory capabilities of anurans because subjects are required to perform behaviors that are suited to their specialized lifestyles. Here we present an assay that further demonstrates that ranids can discriminate between different feeding stimuli and that they might be able to retain such learned events. Our methods were derived from those of Sternthal (1974) except that we used fewer trials per day and did not pretrain the subjects with presentations of edible feeding stimuli before the actual experiment was conducted.

Twelve recently metamorphosed *Rana pipiens* (36.0–42.0 mm SVL) were obtained from Kons Scientific Co. (Germantown, Wisconsin, USA). They were held for several weeks as pairs in 15 × 25 × 40 cm plastic containers with water and paper toweling under a 16L:8D photoperiod at 25 C and fed crickets ad libitum. Before beginning the experiment, the frogs were pretested for their motivation to strike at an artificial feeding stimulus by dangling a small ball of thread a few times in front of each subject. Five frogs responded readily to this pretest and were deemed appropriate for the study. They were placed in separate 30 × 75 cm containers and allowed to acclimate over-