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Allozyme Variation among Disjunct Populations of the Florida Green Watersnake (*Nerodia floridana*)

J. Scott Thompson and Brian I. Crother

The Florida green watersnake, *Nerodia floridana*, has a disjunct distribution in the southeastern United States (Conant and Collins, 1991; Fig. 1). The majority of its range covers Florida, southern Georgia, and southern Alabama; the remainder of its range lies in the southern half of South Carolina. Individuals in the northern segment are uncommon, and as such, the species is currently listed in South Carolina as a Species of Special Concern. Because of this, a genetic comparison of the individuals from northern and southern areas was deemed necessary before future listing considerations (e.g., candidacy for Endangered Species status).

The objective of this study was to examine allozyme variation between the two disjunct populations of *N. floridana*. The null hypothesis is that the two populations are homogeneous in allozyme distribution; the alternative is that they differ. The possible implications of the study are as follows: (1) if the population in South Carolina is diagnosed as a species [following the phylogenetic species concept as the operational form of the evolutionary species concept (Frost and Kluge, 1994)], it should warrant careful consideration for protection under the Endangered Species Act; and (2) if the null is not rejected, that is, no differences in allozyme characters are found, the special status of the species in South Carolina might require reconsideration.

Materials and Methods

Specimens of *N. floridana* were obtained from the northern (n = 4) and southern range (n = 5). Samples of specimens of *Nerodia cyclopion* (n = 10), the putative sister taxon of *N. floridana* (Lawson, 1987), *Nerodia erythrogaster* (n = 10), and *Nerodia fasciata* (n = 10) also were obtained to provide a broader appreciation of genetic variation within *Nerodia*. Sources and disposition of specimens are given Material Examined.

Tissue preparation and electrophoretic conditions essentially followed Murphy et al. (1996). The Appendix details specific loci examined, electrophoretic conditions, and buffer systems. Variation at each locus for each taxon was described by allelic frequencies, average polymorphism, and observed heterozygosity. Total variation among all loci (for each taxon) was measured by total heterozygosity and polymorphism.

Results and Discussion

Allozyme analysis yielded 37 scoreable loci (Table 1). Overall, levels of heterozygosity and polymorphism were low in *N. erythrogaster* (H = 0.003) and *N. fasciata* (H = 0.005). Heterozygotes were observed at *mIcdh-A*, *G3Pdh-A*, and *Pgdh-2*. Average polymorphism across the five taxa was 0.086. Of 37 loci, nine variable loci in *N. fasciata* and four in *N. erythrogaster* exist. No genetic variation was observed within or between northern and southern samples of *N. floridana*.

Results from this study do not reveal allozymic divergence between the disjunct populations of *N. floridana*, and in fact, samples from the two populations were fixed for the same allele at all loci. Lawson (1987) also found no polymorphic loci in a sample of southern *N. floridana*. Observed total heterozygosity for all species of *Nerodia* examined was low (0.00–0.005) relative to other vertebrates (on average about 0.07; Lewontin, 1991) and other reptiles (0.047; Ayala, 1982) but approached levels of heterozygosity found in other snakes (e.g., 0.006–0.089, Dessauer et al., 1987; and 0.036–0.062, Cadle et al.,

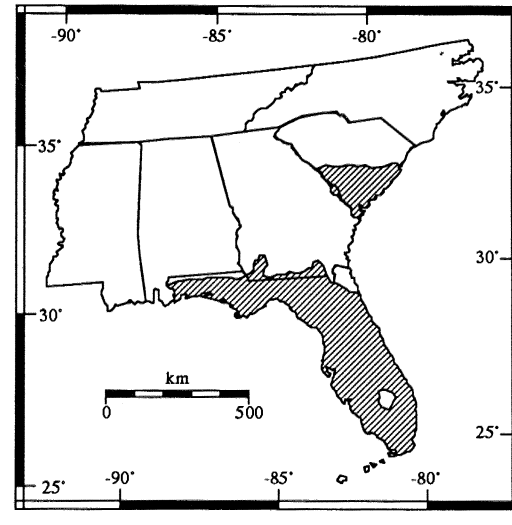

Fig. 1. The approximate distribution of *Nerodia floridana*.

TABLE 1. ALLELE FREQUENCY DISTRIBUTION FOR LOCI THAT VARIED. Enzyme loci are abbreviated on the left. Alleles were scored based on relative mobility.

Locus	Allele	*cyclopion*	*erythrogaster*	*fasciata*	*floridana* (Flo.)	*floridana* (SC)
Ada	a	—	1.000	1.000	—	—
	b	—	—	—	1.000	1.000
	c	1.000	—	—	—	—
mAta-A	a	—	—	—	—	—
	b	1.000	—	—	1.000	1.000
	c	—	0.100	0.400	—	—
	d	—	0.900	0.500	—	—
	e	—	—	0.100	—	—
sAta-A	b	—	1.000	0.667	—	—
	c	—	—	0.333	1.000	1.000
	d	1.000	—	—	—	—
Ck-A	a	1.000		1.000	1.000	1.000
	b	—	1.000	—	—	—
			—			
Dldh-A	a	1.000	—	—	1.000	1.000
	b	—	1.000	—	—	—
	d	—	—	—	—	—
Pep-D	b	—	—	—	1.000	1.000
	d	1.000	—	0.700	—	—
	e	—	—	0.300	—	—
	f	—	1.000	—	—	—
Est-1	b	—	—	—	1.000	1.000
	c	—	1.000	—	—	—
	d	1.000	—	—	—	—
	e	—	—	1.000	—	—
Fum-A	a	1.000	1.000	1.000	—	—
	b	—	—	—	1.000	1.000
Gcdh-A	a	—	—	0.200	—	—
	b	—	0.800	0.700	—	—
	c	—	0.200	0.100	—	—
	d	1.000	—	—	—	—
	e	—	—	—	1.000	1.000
Gpi-A	b	—	1.000	1.000	—	—
	c	1.000	—	—	—	—
	d	—	—	—	1.000	1.000
G3pdh-A	a	—	1.000	0.850	—	—
	b	—	—	0.150	1.000	1.000
	c	1.000	—	—	—	—
Gapdh	a	—	1.000	1.000	1.000	1.000
	b	1.000	—	—	—	—
mIcdh-A	a	1.000	—	—	1.000	1.000
	b	—	0.150	0.750	—	—
	c	—	0.850	0.250	—	—
sIcdh-A	a	—	—	0.400	—	—
	b	1.000	—	0.600	1.000	1.000
	c	—	1.000	—	—	—
mMdph-A (NADP)	a	1.000	0.800	1.000	1.000	1.000
	b	—	0.200	—	—	—
Mpi-A	a	1.000	1.000	1.000	1.000	1.000
	b	—	—	—	—	—

TABLE 1. CONTINUED

Locus	Allele	*cyclopion*	*erythrogaster*	*fasciata*	*floridana* (Flo.)	*floridana* (SC)
Pgm-A	b	1.000	1.000	1.000	1.000	1.000
	c	—	—	—	—	—
Pgdh-1	b	—	1.000	1.000	—	—
	c	1.000	—	—	1.000	1.000
Pgdh-2	b	1.000	1.000	—	1.000	1.000
	c	—	—	1.000	—	—
sSod-A	a	—	1.000	0.800	1.000	1.000
	b	—	—	0.200	—	—
	c	1.000	—	—	—	—
Xdh-A	b	1.000	1.000	0.800	1.000	1.000
	c	—	—	0.200	—	—

1990). Overall polymorphism (0.00–0.24) also was nearly the same as in other studies of snakes (e.g., 0.038–0.296, Dessauer et al., 1987) and was nearly identical to other studies of *Nerodia* (on average 0.10, Dessauer et al., 1987; Lawson, 1987). In a study on *Nerodia harteri*, Rose and Selcer (1989) found no polymorphism at 10 loci in either of two subspecies (later recognized as two species by Densmore et al., 1992).

Low levels of heterozygosity in *Nerodia* may be explained by either of two hypotheses: (1) the behavior and ecology of the members of the genus *Nerodia* may maintain low levels of genetic variation; and/or (2) the sample sizes used were neither sufficiently large nor geographically diverse to assess reliably genomic heterozygosity.

Information accumulated on ecology of *N. floridana* is consistent with the first hypothesis. In a recent study, Seigel et al. (1995) speculated that *N. floridana* may burrow into pond substrate in times of drought instead of migrating to a filled pond. Absence of dispersion at times of drought could lead to population isolation and small effective population size. Whether reluctance to disperse is widespread in the genus will require further study. It is worth noting that the species with the highest heterozygosity, *N. fasciata*, is the most likely to disperse when water sources dry (Seigel et al., 1995). Although small sample size also may explain the low levels of genetic variation found in our study, Lawson's (1987) sample sizes ranged from 17–111 individuals. Consequently, our results would appear to be reliable estimates of genetic variation (or the absence thereof) in the disjunct populations of *N. floridana*.

The absence of divergence between the two disjunct populations of *N. floridana* suggests that the time the two populations have been separated may have been relatively short. Three possibilities could explain the disjunction: (1) *N. floridana* occurs but has not been found in the intermediate area; (2) the population of *N. floridana* in South Carolina is an introduction; or (3) the disjunction is real, but the populations have yet to diverge. There is no evidence to support possiblities 2 and 3, leaving only 1 as tenable.

We suggest that the disjunction may be an artifact. *Nerodia floridana* is rarely captured by hand and is more nocturnal and less inclined to bask than its sister species, *N. cyclopion* (Mount, 1975). This makes individuals of *N. floridana* difficult to sight in the areas of deep reeds and cattails where it is typically found. With the exception of work done in the Okefenokee Swamp, the State of Georgia has not performed extensive herpetological surveys (although one has begun, Jensen and Moulis, 1997). Pending results of the Georgia survey, we believe the disjunct northern population of *N. floridana* should be dropped from federal consideration of protection.

MATERIAL EXAMINED

Nerodia cyclopion: Louisiana, St. John the Baptist Parish, LSUMZ 59055, 59057–61, 59077, 59079, 59081, 59085; *Nerodia erythrogaster*: Louisiana, East Baton Rouge Parish, LSUMZ 59038–39, 59044–47, 59082–84, 59092–93; *Nerodia fasciata*: Louisiana, St. John the Baptist Parish, LSUMZ 59070, 59072–73, 59086–87, 59089; East Baton Rouge Parish, LSUMZ 59067, 59071; Florida, Alachua County, LSUMZ 59088, 59091; *Nerodia floridana*: Florida, Alachua County, *HCD 4408, 4421, 4428, 4451; Bay County, HCD 4548; South Carolina, Berkeley County, LSUMZ 59076, 59080; Aiken County, LSUMZ 59075,

59078. *HCD are tissue numbers from the frozen tissue collection at LSUMZ.

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APPENDIX. SUMMARY OF ELECTROPHORETIC CONDITIONS OF PRESUMPTIVE GENE LOCI IN THIS STUDY.

Enzyme system	Enzyme commission number	Locus	*Assay conditions	Tissue
Aconitate hydratase	4.2.1.3	*mAcon-A*	C	Liver
Aconitate hydratase	4.2.1.3	*sAcon-A*	C	Liver
Adenosine deaminase	3.5.4.4	*Ada*	B	Liver
Aspartate transaminase	2.6.1.1	*mAta-A*	D	Liver
Aspartate transaminase	2.6.1.1	*sAta-A*	D	Liver
Catalase	1.11.1.6	*Cat*	A	Liver
Creatine kinase	2.7.3.2	*Ck-A*	B	Muscle
Dihydrolipoamide dehydrogenase	1.8.1.4	*Dldh-A*	A	Liver
Peptidase-cytosol nonspecific (glycyl-L-leucine)	3.4.13.18	*Pep-A,C,S*	F	Liver
Tripeptide aminopeptidase (L-leucylglycylglycine)	3.4.13.4	*Pep-B*	F	Liver
XAA-PRO dipepetidase (L-phenylalanyl-L-proline)	3.4.13.9	*Pep-D*	F	Liver
Esterase (napthyl acetate) nonspecific		*Est-1*	C	Liver
Esterase (napthyl acetate) nonspecific		*Est-2*	C	Liver
Fumarate hydratase	4.2.1.2	*Fum-A*	C	Liver
Glucose 1-dehydrogenase	1.1.1.47	*Gcdh-A*	C	Liver
Glucose-6-phosphate isomerase	5.3.1.9	*Gpi-A*	A	Muscle
Glutamate dehydrogenase	1.4.1.2	*Gtdh-A*	C	Liver
Glycerol-3-phosphate dehydrogenase	1.1.99.5	*sG3pdh-A*	E	Muscle
Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)	1.2.1.12	*Gapdh*	C	Muscle
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*mIcdh-A*	C	Liver
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*sIcdh-A*	E	Liver
L-Lactate dehydrogenase	1.1.1.27	*Ldh-A*	A	Muscle
L-Lactate dehydrogenase	1.1.1.27	*Ldh-B*	A	Kidney
Malate dehydrogenase (NAD+)	1.1.1.37	*mMdhp-A*	C	Liver
Malate dehydrogenase (NAD+)	1.1.1.37	*sMdhp-A*	C	Liver
Malate dehydrogenase (NADP+)	1.1.1.40	*mMdhp-A*	C	Kidney
Malate dehydrogenase (NADP+)	1.1.1.40	*sMdhp-A*	C	Kidney
Mannose-6-phosphate isomerase	5.3.1.8	*Mpi-A*	A	Muscle
Octanol dehydrogenase	1.1.1.73	*Odh*	A	Liver
Phosphoglucomutase	5.4.2.2	*Pgm-A*	C	Muscle
Phosphogluconate dehydrogenase (decarboxylating)	1.1.1.44	*Pgdh-1*	C	Liver
Phosphogluconate dehydrogenase (decarboxylating)	1.1.1.44	*Pgdh-2*	C	Heart
Purine-nucleoside phosphorylase	2.4.2.1	*Pnp-A*	A	Liver
Superoxide dismutase	1.15.1.1	*sSod-A1*	E	Liver
Superoxide dismutase	1.15.1.1	*sSod-A2*	B	Muscle
Xanthine dehydrogenase	1.1.1.204	*Xdh-A*	A	Liver

* A: "Poulik" system, 11v cm; B: Tris-HCL pH 8.5, 14.3 V/cm 2.5 h; C: Tris-citrate pH 8.0, 6V/cm 6h; D: Lithium hydroxide, 14.3 V/cm 5h; E: Tris-citrate EDTA pH 7.0 9.7 V/cm 10 h.; F: Tris Citrate pH 7.0, 5 V/cm 24h. Slight modifications in voltage or time were employed in some instances to obtain better resolution. Stains were employed following Murphy et al. (1996).