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Genetic Similarity of Shadow and Ozark Basses (*Ambloplites*) as Determined by Mitochondrial DNA Analysis

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The rock basses, *Ambloplites*, contain four nominal species and represent one of eight genera within the sunfish family (Centrarchidae). The shadow bass (*A. ariommus* Viosca) occurs in the southern plains states east and west of the Mississippi River. The range of the shadow bass west of the Mississippi River is limited to Arkansas and parts of southern Missouri and eastern Oklahoma. Several Arkansas river drainages contain the shadow bass including the Red, Ouachita, Arkansas, Illinois, Little Red, Strawberry, Spring, Black, and St. Francis river drainages (Robison and Buchanan, 1988). The Ozark bass (*A. constellatus* Cashner and Suttkus) is found in streams of the White River drainage in northern Arkansas and southern Missouri and in the Buffalo River (Robison and Buchanan, 1988). The geographic ranges of the shadow and Ozark basses are proximal to one another. The Roanoke bass *(A. cavifrons* Cope) is limited to Virginia and North Carolina (Cashner and Jenkins, 1982). Only the rock bass, *A. rupestris* Rafinesque, has an extensive range throughout much of the Mississippi River drainage.

Several morphological and anatomical features distinguish the shadow and Ozark basses including blotchy pigmentation versus irregular rows of spots, a deeper versus a more slender body depth, and the presence of less than or more than 41 lateral line scales (Robison and Buchanan, 1988). Genetic analysis of *Ambloplites* is limited to a single allozyme study. Koppleman et al. (2000) could not genetically distinguish between the shadow and rock basses, yet identified fixed allelic differences at two of 41 loci between the Ozark and shadow basses. No direct DNA comparisons have been performed on this genus. One of our research goals was to perform a genetic comparison of the *Ambloplites* species native to Arkansas (shadow and Ozark basses) using restriction endonuclease digestion of intact mitochondrial DNA (mtDNA). A second goal was to estimate the time of divergence using a commonly used mtDNA molecular clock (Brown et al., 1979).

Shadow bass were collected from the South Fork of the Spring River (n = 22), the Spring River (n = 28), and the Caddo River (n = 2), while Ozark bass (n = 24) were collected from the Buffalo River. Mitochondria and mtDNA were isolated and analyzed from liver tissue using techniques described by Johnson et al. (2002). Purified mtDNA was digested for 7 h at 37° C using 14 restriction endonucleases (*Bam*HI, *Bgl*I, *Bgl*II, *Csp*45I, *Dra*I, *Eco*RI, *Eco*RV, *Mlu*I, *Pst*I, *Pvu*II, *Sal*I, *Sca*I, *Xba*I, *Xho*I), under conditions recommended by the supplier (Promega Corp.). Fragment sizes generated were determined through the use of a least-squares fit program (Schaeffer and Sederoff, 1981). Variables measured included genome size, percentage genome analyzed, nucleon diversity (h; Nei and Tajima, 1981) and nucleotide sequence divergence (Nei and Li, 1979). Times of divergence were estimated from observed levels of sequence divergence using a rate of sequence divergence of 2% per million years (Brown et al., 1979). A phenogram was constructed by the unweighted pair group method (UPGMA; Sokal and Sneath, 1963) using matrices of distance values with NTSYS-PC: Numerical Taxonomy and Multivariate Analysis System (Rohlf, 1990) as well as an inferred phylogenetic tree using the Dollo parsimony algorithm in Phylogeny Inference Package (PHYLIP; Felsenstein, 1993). The northern largemouth bass (*Micropterus salmoides salmoides* Lacepede) and the green sunfish (*Lepomis cyanellus* Rafinesque), basal members of other centrarchid genera, were used for comparison of higher taxonomic relationships (Branson and Moore, 1962; Avise and Smith, 1977).

Mean genome size ranged from 16,676 (± 337 SE) base pairs for shadow bass to 17,170 (± 292 SE) base pairs for Ozark bass. These estimates are similar to values obtained for *Lepomis* (range 16,639 to 16,958; Johnson and Williams, 2003) and slightly lower than that obtained for *Micropterus* (range of 17,346 to 17,779; Johnson et al., 2002).

The total number of restriction fragments generated using 13 restriction endonucleases was 43, with 31 fragments identified for the Ozark bass and 32 fragments for the shadow bass. Three of the restriction endonucleases did not identify recognition sequences within individual species (*Bam*HI, Ozark bass, shadow bass; *Sal*I, shadow bass; and *Xho*I, Ozark bass and shadow bass) [Table 1]. The dominant haplotype (A) for the shadow bass was found in all three populations sampled (Caddo, South Fork and Spring rivers). Two additional haplotypes (B and C) were identified for single individuals from the South Fork (B) and the Spring River (C). Haplotype diversity for shadow bass of the South Fork was 0.15 with a diversity of 0.07 for the Spring River

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Table 1. Composite haplotypes of *A. ariommus, A. constellatus, L. cyanellus* and *M. salmoides* collected from northeast Arkansas. Restriction endonucleases are in columns as follows: *Bam*HI, *Bg*lI, *Bg*lII, *Csp*45I, *Dra*I, *Eco*RI, *Eco*RV, *Mlu*I, *Pst*I, *Pvu*II, *Sal*I, *Sca*I, *Xba*I, and *Xho*I.

Species	Genotype Assignment	n
A. ariommus A	AAAAAAAAAAAAA	50
A. ariommus B	AAAAABAAAAAAA	1
A. ariommus C	AAAAAABAAAAAA	1
A. constellatus	ABAABABBABBBA	24
M. salmoides	BCABCACCBCCCB	1
L. cyanellus	CDACDCDDCBDDC	1

population. Haplotype B had a unique restriction site for the endonuclease *Eco*RV, whereas haplotype C had a unique restriction site for *Mlu*I. There was a single haplotype found within the Ozark bass sampled (h = 0.00). Unique restriction profiles between the Ozark and shadow basses were identified for the following restriction endonucleases: *Bg*lI, *Dra*I, *Pst*I, *Sal*I, *Sca*I, and *Xba*I.

Estimated nucleotide sequence divergence, standard error, and time of divergence for *Ambloplites, Lepomis,* and *Micropterus* are found in Table 2. Divergence values ranged from 0.0612 for the shadow and Ozark basses to 0.3216 for the largemouth bass and green sunfish. The divergence values for the shadow and Ozark basses are lower than that found for congeneric members within the genera *Lepomis* and *Micropterus* (Johnson and Williams, 2003; Johnson et al., 2002). Data indicated that *Ambloplites* is more similar to the largemouth bass than to the green sunfish. Estimated time of divergence for the two species of *Ambloplites* studied was 3.07 million years ago (Table 4). Unfortunately, there is no reported fossil record of *Ambloplites* on which to base a comparison. It must be noted that the molecular clock of 2% per million years has not been consistent through all taxa (Grewe et al., 1990). Additionally, these estimates are based on few populations within each species and therefore the values obtained are at best estimations of the divergence periods between these particular populations rather than the species as a whole.

Construction of a cladogram generated utilizing the Dollo parsimony algorithm program revealed that *Ambloplites* was most similar to *Micropterus* and less so to *Lepomis* (Fig. 1). A UPGMA dendrogram derived from the average genetic distances between species was consistent with that above. A cophenetic correlation of 0.76 was

Table 2. Mitochondrial DNA sequence divergence (above diagonal) and estimated time of divergence in millions of years (below diagonal) for *Ambloplites, Lepomis* and *Micropterus* studied. Standard errors of the mean in parentheses beneath the means.

Species	1	2	3	4
1. *A. ariommus*	***	0.0615	0.2044	0.1675
		*** (0.0014)	(0.0003)	(0.0030)
2. *A. constellatus*	3.07	***	0.2121	0.1371
	(0.01)		*** (0.0004)	(0.0006)
3. *L.cyanellus*	10.22	10.66	***	0.3216
	(0.01)	(0.01)		*** (0.0011)
4. *M. salmoides*	8.37	6.86	16.08	***
	(0.01)	(0.01)	(0.01)	***

obtained, indicative of a suitable fit between the tree generated and the data matrix (Rohlf, 1990). In contrast, Avise and Smith (1977) hypothesized that *Lepomis* was intermediate to *Micropterus* and *Ambloplites* based upon allozyme analysis of several centrarchid genera. However, Avise and Smith (1977) sampled the rock bass as compared to the shadow and Ozark basses, and they used the average genetic distances of ten *Lepomis* species rather than looking at a specific species (green sunfish). Future study of the genus *Ambloplites* at the population, subspecies, and species levels is required to further our understanding of the historical biogeography of this genus. Additional population data may alter the proposed relationships of these taxa.

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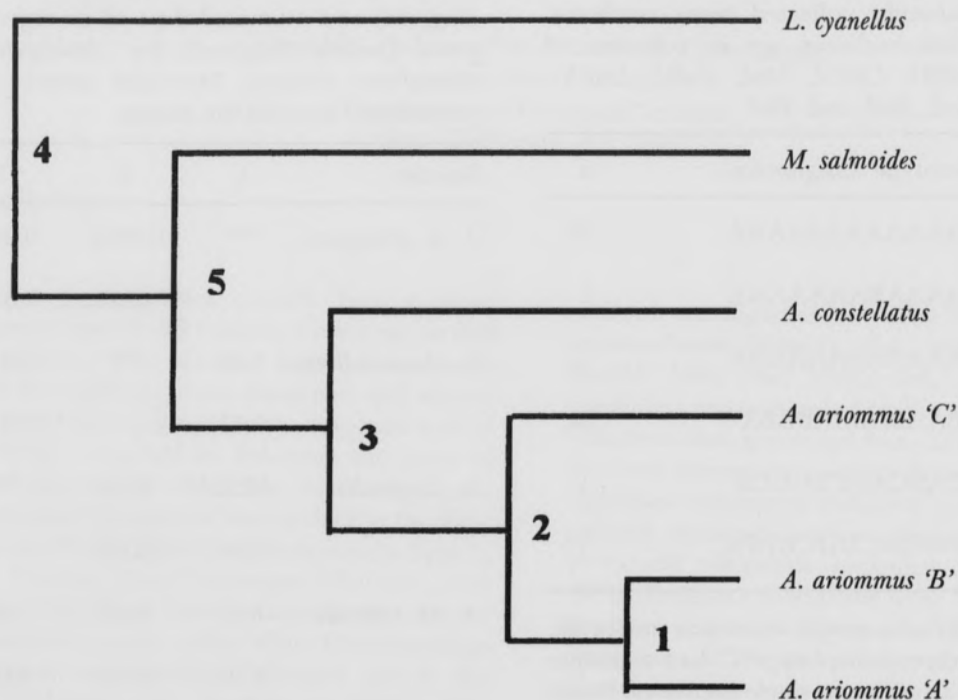
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Fig. 1. Cladogram of character state matrices for presence/absence of presumptive restriction sites utilizing the Dollo parsimony algorithm. One parsimonious tree (22 steps) was generated.

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