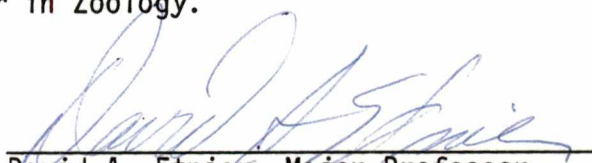
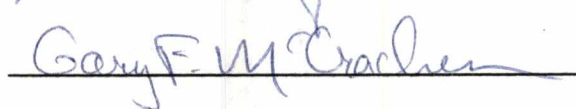


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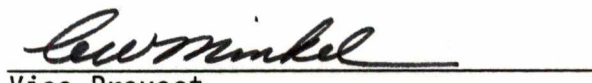
I am submitting herewith a thesis written by Richard Gene LeDuc entitled "Morphological and Biochemical Variation in the Bluntnose Shiner, Notropis camurus (Cyprinidae)." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.


David A. Ethnier, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Vice Provost
and Dean of The Graduate School

MORPHOLOGICAL AND BIOCHEMICAL VARIATION IN THE BLUNTFACE

SHINER, Notropis camurus (Cyprinidae)

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Richard Gene LeDuc

December 1984

To C. C., with love.

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ABSTRACT

Patterns of variation in morphology were compared to those determined by biochemical analysis for populations of Notropis camurus. Morphological divergence between two races of N. camurus was most evident in the character of body form and was great enough to warrant subspecific status for the two races. However, analysis of 46 alleles at 20 loci showed a pattern of variation discordant with that of morphology. This precludes any taxonomic decision in the absence of further analysis. Evidence for gene duplication and rediploidization was seen at the MDH1 locus. Electrophoretic data also support previous conclusions based on morphology about the close affinity between N. camurus and N. galacturus.

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INTRODUCTION

Despite a long and varied taxonomic history, the subgenus Cyprinella represents one of the more coherent assemblages of species within the diverse genus Notropis (Cyprinidae) (Gibbs, 1957a). In a series of papers, Gibbs (1957a, b, c, 1961, 1963) examined the subgenus in its entirety as well as the several species complexes recognized within it. Although broad in scope and the most comprehensive look at the subgenus to date, these papers left some unanswered questions about at least one of the species with regard to its intra-specific variation and its relationships to other Cyprinella.

The bluntface shiner, Notropis camurus (Jordan and Meek), has a disjunct distribution divided into populations east and west of the Mississippi River. The western populations are found in tributaries to the Arkansas River in Arkansas, Kansas, Missouri, and Oklahoma while in the east it is found in direct tributaries to the Mississippi River from Louisiana to Kentucky as well as some tributaries to the lower Tennessee River in West Tennessee (Figure 1, copied from Gilbert and Burgess, 1980). In his treatment of the species, Gibbs (1961) recognized two races of N. camurus, designating the eastern form as the lower Mississippi race and the western form as the Arkansas race. However, his lower Mississippi race was based on individuals collected from no farther north than the Yazoo River in Mississippi and no specimens were examined from Mississippi River tributaries in West Tennessee, such as the Obion, Forked Deer, Hatchie, and

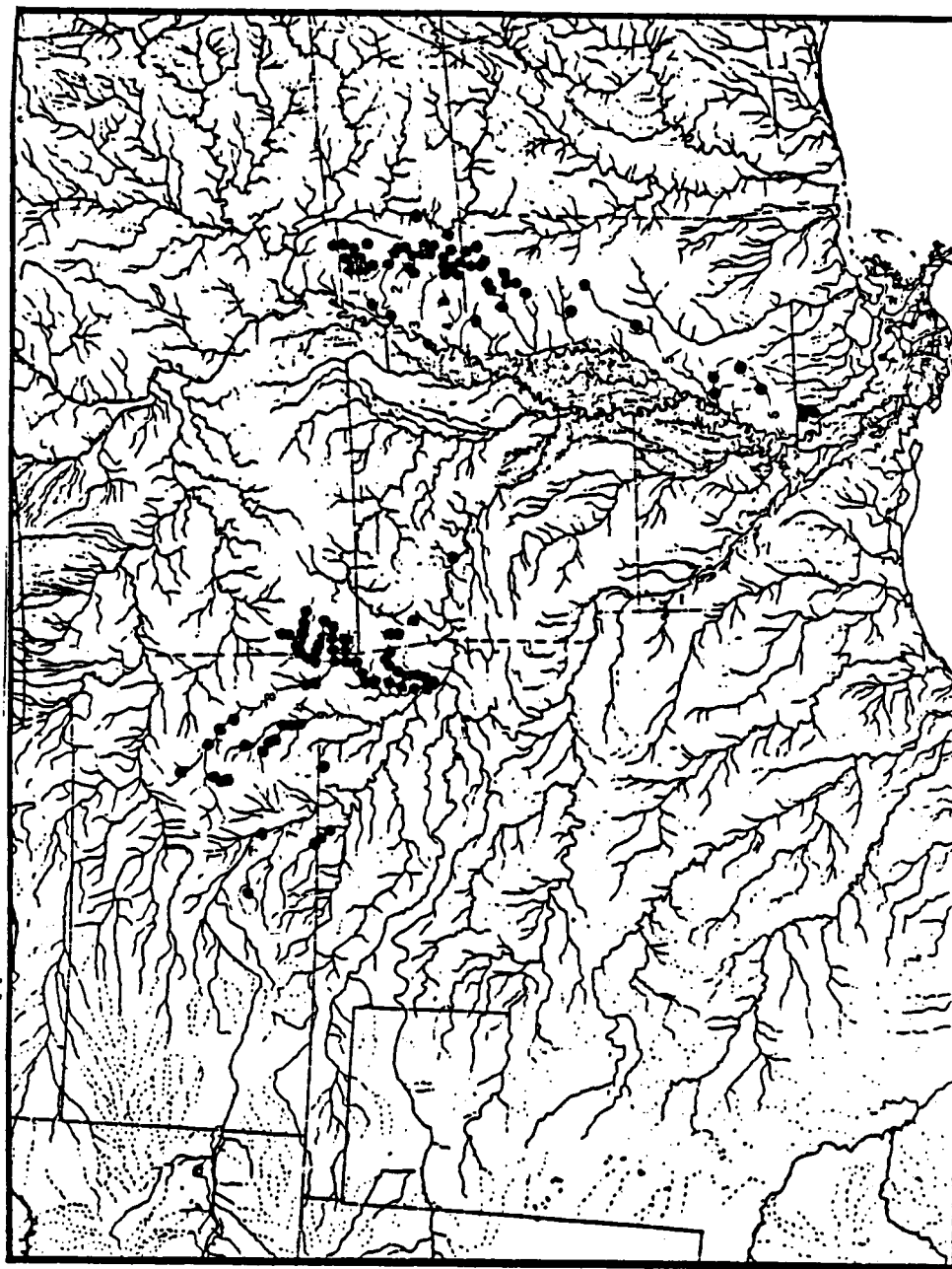


Figure 1. Range of the bluntface shiner, *Notropis camurus*, copied from Gilbert and Burgess (1980). Drainages examined in this study are as follows: 1-Obion R. 2-Forked Deer R. 3-Hatchie R. 4-Loosahatchie R. 5-Yazoo/Big Black R. 6-Homochitto/Buffalo R. 7-Upper Arkansas R. 8-Neosho R. 9-Fall R.

Loosahatchie Rivers. Therefore, the designation of the two races of N. camurus failed to include a major part of that species range east of the Mississippi River.

In his paper on the N. whipplei complex, Gibbs (1963) utilized three specimens of N. whipplei from "lower Mississippi tributaries in Tennessee and Mississippi." Whether they are misidentifications of N. camurus, errant introductions, or bona fide range extensions, these localities are outside the normal range of N. whipplei given by Gilbert and Burgess (1980) but are in rivers where N. camurus is common.

Gibbs (1961) distinguished the two races of N. camurus partly on the basis of lateral-line scale counts, in which the lower Mississippi race tended toward a higher count, with considerable overlap. He also noted that the Arkansas race tended toward a less streamlined body form than the lower Mississippi race as indicated by greater caudal peduncle depth and dorsal origin-lateral line measurements, expressed as percent standard length. His measurements were only on fish between 50 and 60 mm standard length (SL), presumably to avoid sample variation caused by allometric growth. Allometric growth, whereby different body parts or measurements show different growth rates (Mayr, 1969), could cause the measured proportions to differ for different size fish. For example, a minor divergence in characters seen in fish 50-60 mm SL may become a significant difference between adults of 70-100+ mm SL.

One purpose of this study is to reexamine the variation in N. camurus for the divergent morphological characters, expanding both the range of sizes of individuals measured as well as the geographic range of localities. Divergence between the eastern and western forms in total ranges is poorly understood and the possibility that the races merit subspecies recognition exists.

Another question concerns the relationship of N. camurus to other members of the subgenus Cyprinella. Gibbs (1957a, 1961) considered N. galacturus (Cope), the whitetail shiner, to be its closest relative based mainly on shared pigmentation and breeding tubercle patterns. It has been suggested, however, that N. camurus may be more closely related to the steelcolor shiner, N. whipplei (Girard) (Gilbert and Burgess, 1980; Etnier, pers. comm.), which Gibbs (1957a, 1963) placed in a different species complex within the subgenus, but to which N. camurus has a great resemblance. For individuals other than breeding males, distinguishing N. camurus from N. whipplei is difficult and based mainly on the blunter snout and depigmented caudal base of N. camurus (Gibbs, 1961). However, the snout bluntness is less extreme in the lower Mississippi race (Gibbs, 1961) and the depigmented caudal base is often vague and difficult to see in West Tennessee specimens (Etnier, unpublished). N. camurus is mostly allopatric with N. whipplei and N. galacturus, but is easily distinguished from the latter species by a variety of characters (Gibbs, 1961).

Therefore, it appears that intra- and interspecific relationships of N. camurus are still somewhat questionable and warrant further

study. In addition to morphological data, biochemical analysis by starch-gel electrophoresis has become a useful tool for examining problems in systematics (Avisé, 1974). The process has been used for other cyprinids such as some western genera (Avisé and Ayala, 1976; Avisé et al, 1975), the genus Campostoma (Buth and Burr, 1978), and the troublesome subgenus Luxilus of Notropis (Rainboth and Whitt, 1974; Menzel, 1976; Buth, 1979; Buth and Mayden, 1981; Dowling and Moore, 1984). While certainly not unequivocal, patterns of variation and relationships determined by electrophoresis can be compared and contrasted to those established by classical taxonomic methods (Buth, 1984). Concordance between the methods provides a strong basis for taxonomic decisions while dissimilarities, such as in Avisé et al (1975), can indicate where further study is needed. In this study, reexamination of morphological data will be accompanied by a biochemical analysis by starch-gel electrophoresis to attempt a better understanding of the bluntface shiner.

CHAPTER I

MATERIALS AND METHODS

Preserved fish were provided by the following institutions: University of Kansas Museum of Natural History (Kansas), University of Michigan Museum of Zoology (Kansas and southern Mississippi), and University of Tennessee, Knoxville (West Tennessee, Mississippi, and Oklahoma). A complete list of series examined can be found in Appendix A.

Measurements of standard length, caudal peduncle depth (CPD), and dorsal origin-lateral line (DO-LL) were taken on preserved fish larger than 50 mm SL. Measurements were taken to the nearest 0.5 mm following the methods described in Hubbs and Lagler (1947), as were lateral-line scale counts. Additional counts and measurements taken are given in Appendix B. All measurements are expressed as a percent standard length.

Frozen specimens for electrophoresis from the Buffalo River in southern Mississippi were provided by the University of New Orleans and those from the upper Arkansas River locality (Chikaskia R.) in Kansas by the museum at the University of Kansas. All other specimens for electrophoresis were collected in Tennessee, Mississippi, and Kansas. Fish were collected with a 12 ft. seine, then wrapped in aluminum foil and immersed while still alive in liquid nitrogen. Exceptions were specimens of Notropis galacturus and N. whipplei,

where dry ice was used for freezing. Upon return to the laboratory, the fish were transferred to a deep freezer (-80°C) until processing; a period not exceeding 4 months. Processing and electrophoresis procedures are given in Appendix C.

Electrophoretic samples (species, river system, and number of specimens) were as follows: N. camurus--Obion (36), Forked Deer (30), Hatchie (21), Loosahatchie (37), Big Black (33), Buffalo (14), upper Arkansas (30), Neosho (32), Fall (33); N. galacturus--upper Tennessee (22); and N. whipplei--upper Tennessee (11). A total of 46 alleles at 20 loci on 4 buffer systems were examined on all fish (Table 1). After the gels were scored and allele frequencies tabulated, genetic similarity coefficients (I) were calculated according to Nei (1972). These I values were used to derive a phenogram of genetic relationships using the unweighted pair-group method with arithmetic means (UPGMA). A similar treatment using a modified identity value (Hillis, 1984) was also calculated (Appendix D).

Table 1. Allozymes Examined and Number of Alleles Resolved at Each Locus

Allozyme	E.C. Number	Locus	Number Alleles Resolved	Buffer System
Creatine kinase	2.7.3.2	CK	2	Poulik
Esterase	3.1.1.1	EST	3	Poulik
General protein		GP1	1	LiOH
		GP2	1	LiOH
		GP3	1	LiOH
		G-6-PD	2	TEB
Glucose-6-phosphate dehydrogenase	1.1.1.49			
Lactate dehydrogenase	1.1.1.27	LDH1	1	Histidine
	1.1.1.27	LDH2	2	Histidine
Malate dehydrogenase	1.1.1.37	MDH1	5	Histidine
Mannose phosphate isomerase	5.3.1.8	MPI1	3	Histidine
	5.3.1.8	MPI2	2	Histidine
Octanol dehydrogenase		ODH1	3	TEB
		ODH2	2	TEB
Peptidase	3.4.11 or 13	PEP1	3	LiOH
	3.4.11 or 13	PDP2	2	LiOH
Phosphoglucoisomerase	5.3.1.9	PGI1	4	TEB
	5.3.1.9	PGI2	3	TEB
Phosphoglucumutase	2.7.5.1	PGM	3	LiOH
Superoxide dismutase	1.15.1.1	SOD	1	TEB

Note: Stain and buffer recipes are given in Appendix C.

CHAPTER II

RESULTS

Measurements for dorsal origin-lateral line and for caudal peduncle depth are given in Tables 2 and 3, respectively. Values for fish in the 50-60 mm SL class generally agree with those given by Gibbs (1961). It can be seen that body depth as measured by the dorsal origin-lateral line/standard length ratio increases with length of the fish. Furthermore, the western populations (Neosho, Fall, upper Arkansas in Table 2) show a greater increase with size for this proportion than the eastern populations. Therefore, a minor difference in body depth seen in medium-sized fish (50-59.5 mm SL) increases for adults of over 70 mm SL. This is shown graphically in Figure 2. Figure 3 shows a similar change in comparative values for the caudal peduncle depth/standard length ratio for different sized fish. Table 4 presents the lateral-line scale counts for fish from each drainage. They generally agree with those given by Gibbs (1961) for both the Arkansas and lower Mississippi races. There was no noticeable difference in scale counts related to the size of the fish.

For the electrophoretic analysis, Table 5 gives allele frequencies for each polymorphic locus for each population. Fifteen of the 20 loci examined showed polymorphism, although no more than 10 loci were polymorphic in any one population. One locus, MDH1, presents

Table 2. Frequency Distribution of Dorsal Origin-Lateral Line for Notropis camurus, Expressed as Percent Standard Length

River system	mm SL	dorsal-origin lateral line/standard length											
		14	15	16	17	18	19	20	21	22	23		
<u>East</u>													
Tennessee	70+		1	5			1						
	60-69.5		2	1	1								
	70+		3	3	15	8	3	3					
	60-69.5	1	3	11	9	3							
	55-59.5		7	6	3								
Obion	50-54.5	2	6	12	5								
	70+			7	9	4	5	1					
	60-69.5			6	12								
	55-59.5			3	2								
	50-54.5		2	8									
Forked Deer	70+												
	60-69.5												
	55-59.5												
	50-54.5												
Hatchie	70+		3	15	23	19	4						
	60-69.5		9	28	16	5							
	55-59.5		8	26	6	1							
	50-54.5	4	17	17	12	1							
Loosahatchie	70+												
	60-69.5		1	4	7	2	2						
	55-59.5			3	10	3							
	50-54.5		1			1							

Table 2 (Continued)

River system	mm SL	dorsal origin-lateral line/standard length											
		14	15	16	17	18	19	20	21	22	23		
Yazoo/Big Black	70+			1	4	4	3	1					
	60-69.5			5	4	5	2						
	55-59.5		3	3	5	4	1						
	50-54.5		3	6	6	6	1						
Homochitto	70+				3	4	1						
	60-69.5		1	6	4	1							
	55-59.5		2	10	7	3							
	50-54.5		4	24	10	1							
<u>West</u>													
Fall	60-69.5							1	3	1			
	55-59.5							1					
	50-54.5				1		1						
Neosho	70+												
	60-69.5				2	3	3	6	2	3			
	55-59.5			1	4	7	5	2					
	50-54.5			2	4	5	2						
Upper Arkansas	70+								1		2		
	60-69.5		2	1									
	50-54.5		1		1								

Table 3. Frequency Distribution of Caudal Peduncle Depth for Notropis camurus, Expressed as Percent Standard Length

River system	mm SL	caudal peduncle depth/standard length						
		9	10	11	12	13	14	15
<u>East</u>								
Tennessee	70+			2	4	1		
	60-69.5			1	3			
Obion	70+		2	17	12	4		
	60-69.5		2	19	5	1		
	55-59.5		2	10	4			
	50-54.5		2	7	15	1		
Forked Deer	70+			15	10	1		
	60-69.5	1		7	9	1		
	55-59.5				5			
	50-54.5			3	7			
Hatchie	70+		2	14	39	9		
	60-69.5		1	11	38	8		
	55-59.5			9	23	9		
	50-54.5		3	12	30	6		
Loosahatchie	70+			6	7	3		
	60-69.5			2	14			
	55-59.5			1				
	50-54.5				2			
Yazoo/Big Black	70+			2	8	3		
	60-69.5			5	7	4		
	55-59.5			2	10	3	1	
	50-54.5		2	3	13	4		
Homochitto	70+			4	4			
	60-69.5		2	4	7			
	55-59.5		6	12	4			
	50-54.5		7	22	10	1		
<u>West</u>								
Fall	60-69.5					1	4	
	55-59.5					1		
	50-54.5				2			

Table 3 (Continued)

River system	mm SL	caudal peduncle depth/standard length						
		9	10	11	12	13	14	15
Neosho	70+				2	10	3	2
	60-69.5				7	8	1	
	55-59.5			4	2	3	3	
	50-54.5			2	4	4		
Upper Arkansas	70+						2	1
	60-69.5				1	1	1	
	50-54.5				2			

Table 4. Frequen

River System	PGP		MDH 1					MDH 2		MPI 1			MPI 2		LDH 2	
	A	B	A	B	C	D	E	A	B	A	B	C	A	B	A	B
Obion		.85	.18	.50	.32			1.00		.08	.92		1.00			
Forked Deer	.12	.88		.65	.35			1.00			1.00		1.00			1.00
Hatchie		1.00	.24	.55	.21			1.00			1.00		1.00			1.00
Loosahatchie		1.00	.10	.70	.20			1.00			1.00		1.00			1.00
Big Black		1.00	.29	.66	.05			.59	.41		1.00		1.00			1.00
Buffalo		1.00				1.00		1.00			1.00		.95	.05		1.00
Upper Arkansas		1.00				1.00		1.00			1.00		1.00			1.00
Neosho	.08	.91				1.00		1.00			1.00		1.00			1.00
Fall		1.00				1.00		1.00			1.00		1.00			1.00
<u>H. galeatus</u>		1.00					1.00	1.00			1.00		1.00			1.00
<u>H. whipplei</u>		1.00			.05	.95		1.00			1.00		1.00		.86	.14

Note: Alleles at fre

Table 5. Frequency Distribution of Lateral-Line Scale Counts for Notropis camurus

River system	34	35	36	37	38	39	40	N	$\bar{x}$	SD
<u>East</u>										
Tennessee				7				11	36.64	.50
Obion	1	1	4	67	35	3		133	37.08	.80
Forked Deer			16	49	29	1	1	96	37.19	.76
Hatchie		2	60	140	35	2		239	36.90	.67
Loosahatchie			1	11	17	7		36	37.83	.77
Yazoo/Big Black		2	38	75	26	1		142	36.90	.73
Homochitto		7	61	128	16	2	1	215	36.76	.72
<u>West</u>										
Fall	3	4	10	6				23	35.83	.98
Neosho	6	30	76	24	1			137	35.88	.77
Upper Arkansas	1	10	9					20	35.40	.60
East total	1	12	206	477	158	16	2	872	36.96	
West total	10	44	95	30	1			180	35.82	

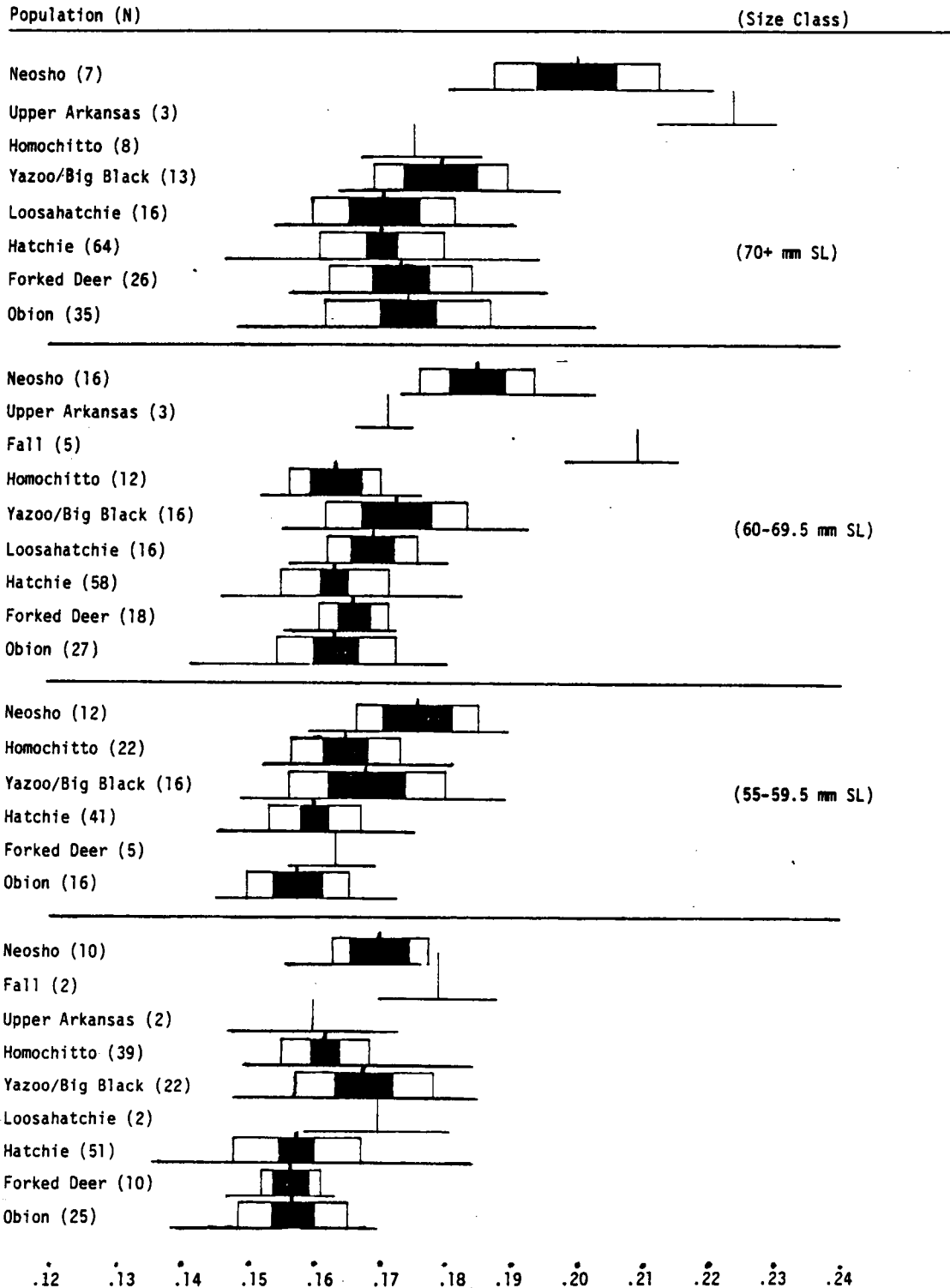


Figure 2. Graphic analysis of dorsal origin-lateral line for Notropis camurus, expressed as percent standard length. Horizontal line=range; vertical line=mean; open rectangle=one standard deviation on each side of mean; black rectangle=two standard errors on each side of mean. (Hubbs and Hubbs, 1953).

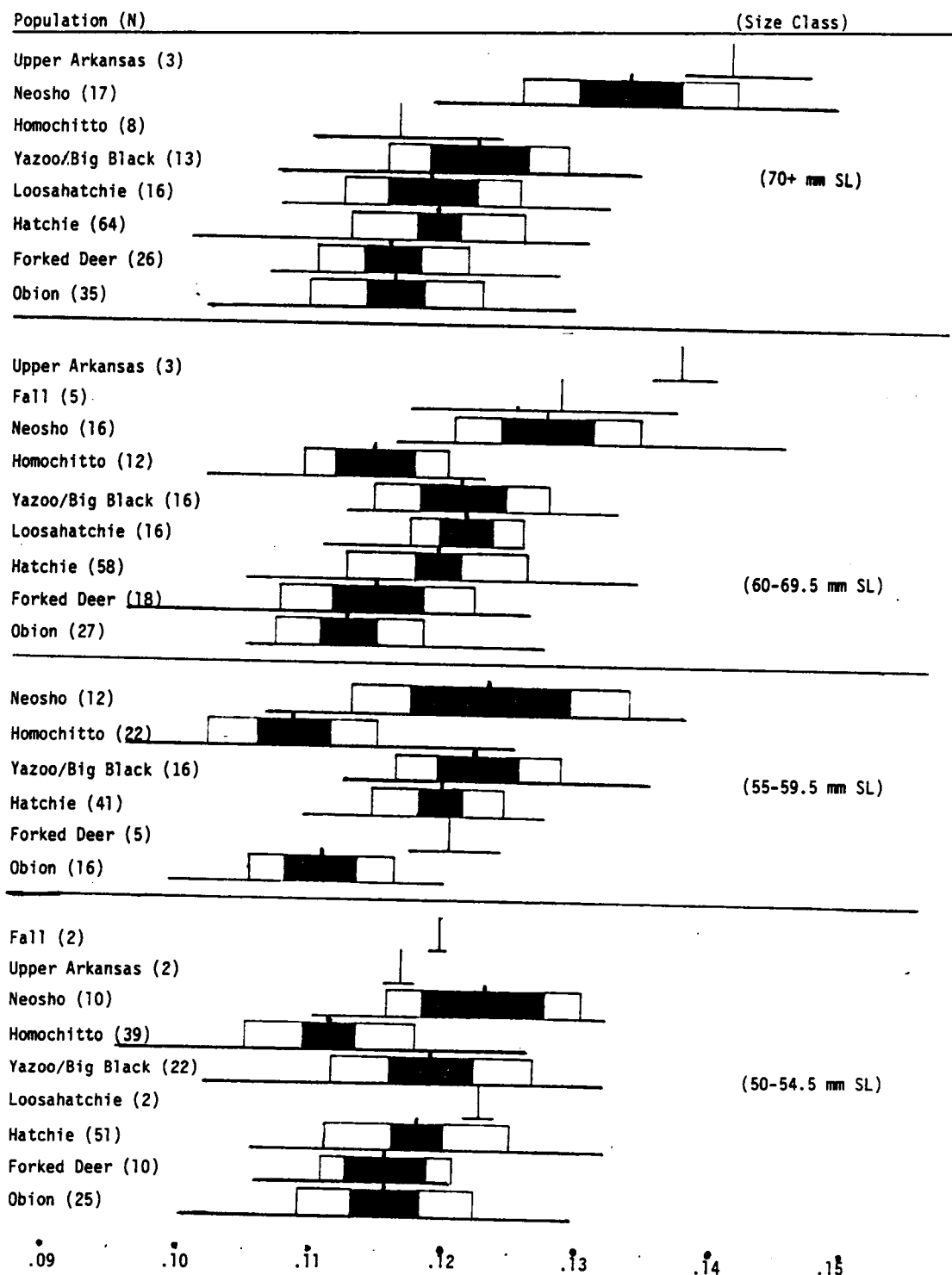


Figure 3. Graphic analysis of caudal peduncle depth for Notropis camurus, expressed as percent standard length. Horizontal line=range; vertical line=mean; open rectangle=one standard deviation on each side of mean; black rectangle=two standard errors on each side of mean (Hubbs and Hubbs, 1953).

an unusual situation and difficulties in scoring. For this reason, I values are calculated both including and excluding the MDH1 locus, and are given in Tables 6 and 7, respectively. Figure 4 depicts a phenogram of genetic relationships calculated using I and the UPGMA over all 20 loci. Figure 5 shows a similar phenogram calculated without the MDH1 locus. Tables 6 and 7 also give the average frequency of heterozygotes (H) over all loci for each population. For analysis of the genetics of the MDH1 locus, see the discussion section.

Rare alleles (frequency less than 0.05) were not listed in Table 5 but were seen in the following populations: Obion (PGM-A, PGI1-D, PGI2-A, G-6-PD-A), Forked Deer (PGI2-C, MDH1-A, MPI1-A), Hatchie (MPI1-A), Loosahatchie (PGI1-D, PGI2-A, MPI2-B), Big Black (PGI1-C, G-6-PD-A, MPI1-C), Buffalo (PGI2-A, ODH1-A, MDH1-C) upper Arkansas (ODH1-C), Neosho (PEP1-A), Fall (PGM-C, PEP1-C). Figure 6 is a photograph of typical gels with alleles and assigned genotypes indicated.

The average observed heterozygosity levels (Tables 6 and 7) fall within the range given by Avice (1977) for various Notropis species, including many in the subgenus Cyprinella. While N. camurus was not examined in his study, his value for N. galacturus ($0.48 \pm .027$) is close to that found in this study (.030) and his value for N. whipplei ($0.069 \pm .034$) is virtually identical to my results (.068).

Table 6. Matrix of Nei's (1972) Genetic Similarity (above diagonal) and Distance (below diagonal) Values Between Populations Examined in This Study Over All 20 Loci

Population	1	2	3	4	5	6	7	8	9	10	11
1. Obion		.990	.979	.981	.972	.956	.932	.932	.930	.953	.818
2. Forked Deer	.010		.982	.985	.963	.947	.917	.918	.915	.941	.794
3. Hatchie	.021	.018		.997	.961	.933	.898	.898	.896	.919	.769
4. Loosahatchie	.019	.015	.003		.967	.937	.907	.908	.906	.922	.772
5. Big Black	.029	.038	.039	.034		.929	.901	.901	.900	.918	.787
6. Buffalo	.045	.054	.069	.065	.074		.986	.986	.985	.944	.852
7. Upper Arkansas	.071	.087	.108	.097	.105	.014		.999	.997	.929	.834
8. Neosho	.070	.086	.108	.097	.104	.014	.001		.999	.930	.830
9. Fall	.073	.089	.110	.098	.106	.015	.033	.001		.928	.829
10. <u>N. galacturus</u>	.048	.060	.085	.081	.086	.058	.073	.073	.075		.809
11. <u>N. whipplei</u>	.201	.231	.263	.258	.240	.161	.182	.187	.187	.213	
H	.132	.119	.090	.082	.015	.064	.012	.014	.021	.030	.068

Note: All populations are of N. camurus unless otherwise indicated. Mean heterozygosity levels over all loci (H) are given for each population.

Table 7. Matrix of Nei's (1972) Genetic Similarity (above diagonal) and Distance (below diagonal) Values Between Populations Examined in This Study Over All Loci Exclusive of MDH1

Population	1	2	3	4	5	6	7	8	9	10	11
1. Obion		.991	.979	.983	.974	.991	.967	.968	.965	.989	.848
2. Forked Deer	.009		.984	.985	.966	.986	.956	.957	.954	.982	.827
3. Hatchie	.021	.016		.998	.962	.968	.932	.932	.930	.954	.797
4. Loosahatchie	.018	.015	.002		.968	.976	.945	.946	.944	.961	.804
5. Big Black	.026	.035	.039	.033		.968	.939	.940	.938	.958	.820
6. Buffalo	.009	.014	.033	.025	.033		.985	.986	.984	.994	.844
7. Upper Arkansas	.034	.045	.071	.056	.063	.015		.999	.997	.980	.825
8. Neosho	.033	.044	.070	.056	.062	.014	.002		.999	.980	.820
9. Fall	.036	.047	.073	.057	.064	.016	.003	.001		.978	.820
10. <u>N. galacturus</u>	.011	.018	.047	.040	.043	.006	.021	.020	.223		.852
11. <u>N. whippiei</u>	.165	.190	.226	.218	.198	.170	.192	.198	.198	.160	
H	.104	.105	.058	.067	.128	.064	.012	.015	.022	.031	.067

Note: All populations are of N. camurus unless otherwise indicated. Mean heterozygosity levels over 19 loci (H) are given for each population.

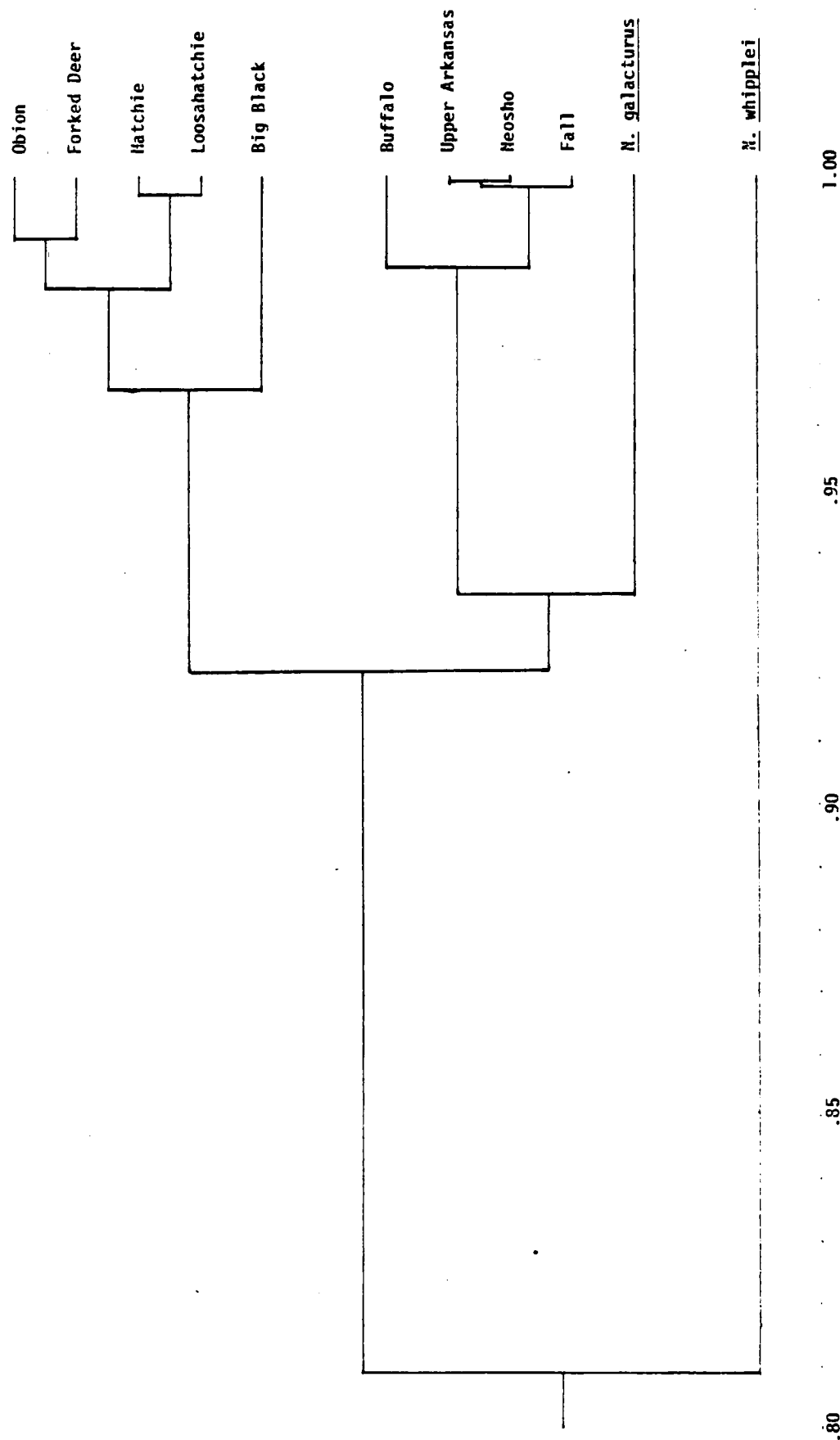


Figure 4. Phenogram of genetic similarity calculated using Nei's (1972) I value and the unweighted pair-group method with arithmetic means (UPGMA) for 20 loci. Populations are of Notropis camurus unless otherwise indicated.

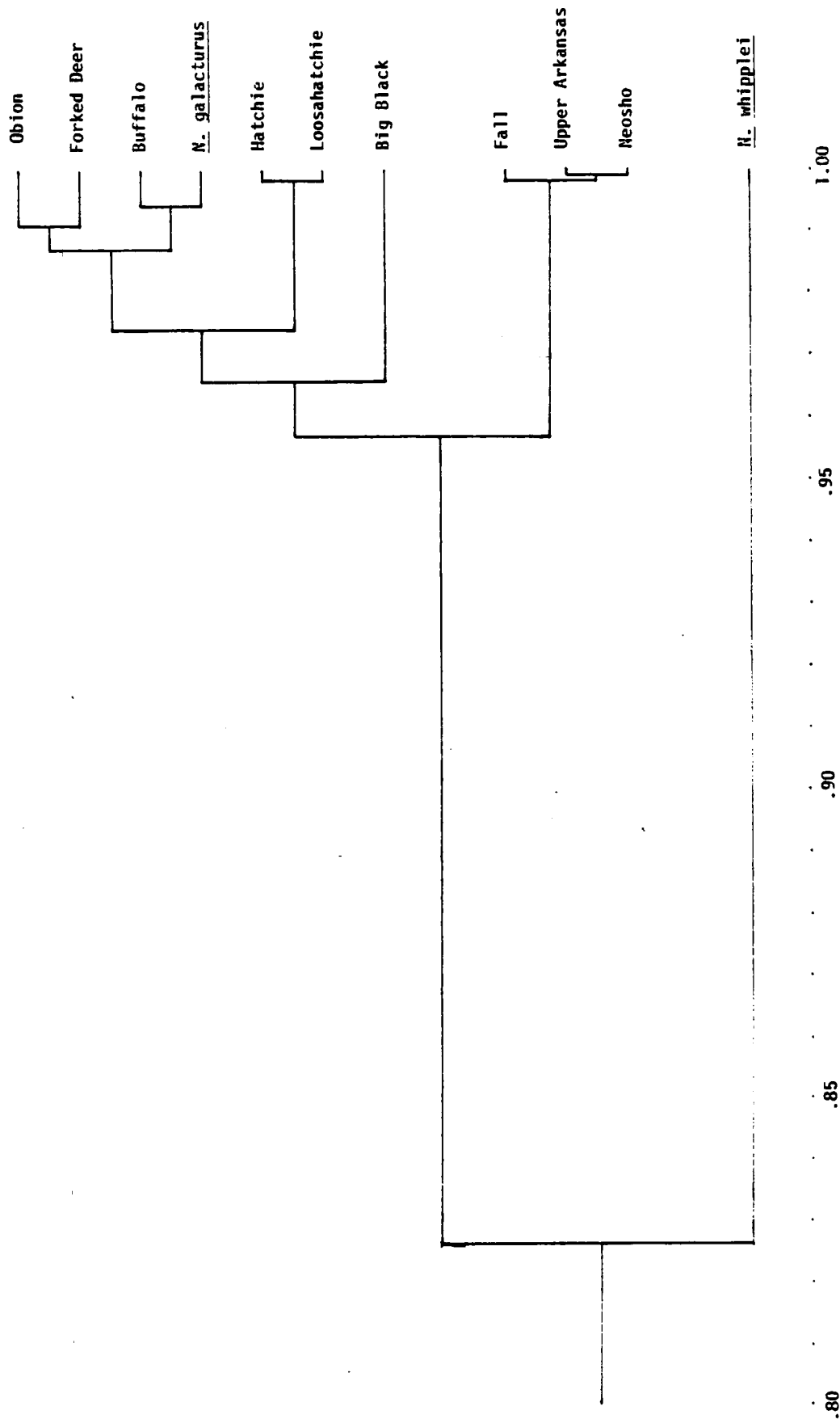


Figure 5. Phenogram of genetic similarity calculated using Nei's (1972) I value and the unweighted pair-group method with arithmetic means (UPGMA) for all loci exclusive of MDH1. Populations are of Notropis camurus unless otherwise indicated.

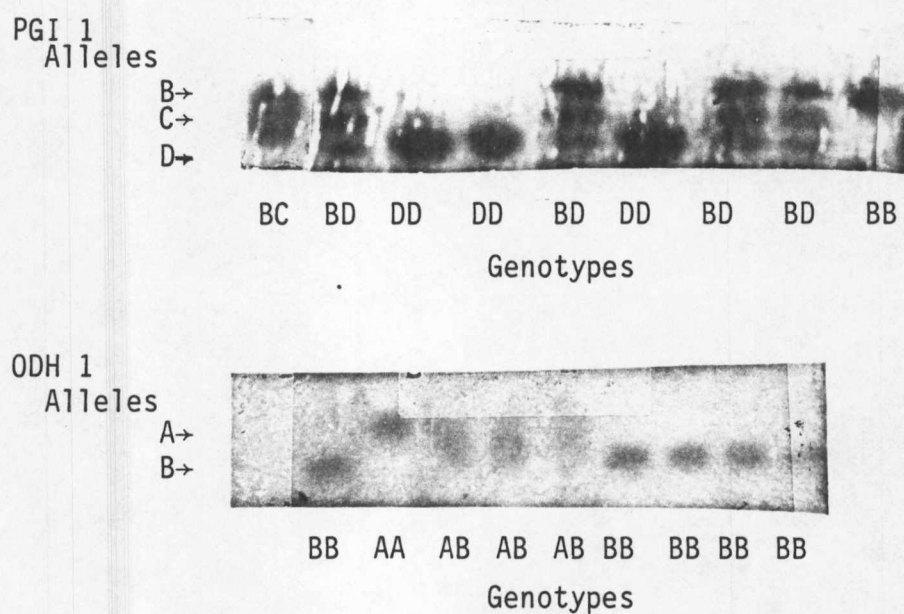


Figure 6. Photographs of allozyme stains at the PGI1 and ODH1 loci, with alleles and assigned genotypes indicated.

CHAPTER III

DISCUSSION

Morphology

The morphological analysis of Notropis camurus (Tables 2-4, pp. 10-14; Figures 2 and 3, pp. 15 and 16) indicate that considerable divergence has taken place between eastern and western populations. While lateral-line scale counts overlap substantially (Table 4), body form has diverged, at least in larger fish, to little or no overlap (Figures 2 and 3). This supports elevating the Arkansas and lower Mississippi races to the level of subspecies. This divergence is greater than that detected by Gibbs (1961) in establishing the two races and is even greater than the divergence on which he based naming of subspecies in N. spilopterus (Gibbs, 1957b) and N. venustus (Gibbs, 1957c). Another consideration is the difference in habitat selection shown by the two forms. Cross (1967) states that in Kansas, N. camurus is rarely found over mud or sand substrates. Pfleiger (1975) states that it favors bottoms of gravel or rubble in Missouri. On the other hand, Bailey and Taylor (1950) describe the lower Mississippi form as occurring over sandy bottoms. Though it was not determined if this may reflect habitat availability and/or the effects of interspecific competition, my collections suggest similar habitat preferences for both regions. Tennessee and Mississippi fish were invariably collected over sandy substrates while Kansas fish were taken over rock or gravel.

The morphological data collected suggest that the two forms may qualify for subspecies recognition. Methods of quantification of divergence for the purpose of objectivity are many and varied (see Mayr, 1969 for a brief summary). To use one approach, Mayr's (1969) calculation of the coefficient of difference (CD) can be applied. The CD between two populations is determined by dividing the difference in their means for a particular character by the sum of their standard deviations. He proposed a CD level of at least 1.28 for subspecies recognition. This corresponds approximately to the widely-used 75-percent rule, whereby 75 percent of the individuals of one population differ from all (97 percent) of the individuals of the other population. Between the eastern and western forms of N. camurus, the CD varies from 0.76 (Neosho-Yazoo) to 1.29 (Neosho-Forked Deer) for CPD and from 0.92 (Neosho-Yazoo) to 1.36 (Neosho-Hatchie) for DO-LL between populations. In addition, the disjunct nature of their respective ranges and the absence of any apparent cline in the characters examined fit well with the criteria put forth by Mayr (1969) and Simpson (1961) for the recognition of subspecies.

Electrophoresis

As stated in the results section, the MDH1 locus warrants special concern. In eastern populations south to the Big Black River, this enzyme stains as a single polymorphic band, with three alleles being distinguishable. However, in the western populations as well as those from extreme southern Mississippi (Buffalo R.), MDH1 invariably

stains as a double band. In mobility, this double band appears identical to the AB heterozygote which occasionally appeared in West Tennessee populations (Figure 7). The fact that all individuals in some populations stained in such manner presents a problem in interpretation. A population of 100% heterozygotes is in violation of Hardy-Weinberg equilibrium and is virtually impossible according to Mendelian genetics.

One possible explanation of the situation is that of gene duplication, whereby multiple copies of a gene are produced (Buth, 1983). If one of the copies then undergoes a mutation and produces an enzyme of different mobility, apparent heterozygosity results. In actuality, there are two separate loci now fixed for different alleles. Such duplication has been noted in a number of fishes, such as salmonids (Allendorf and Utter, 1973); Bailey et al, 1970), catostomids (Ferris and Whitt, 1977a), and cobitids (Ferris and Whitt, 1977b). However, as Buth (1983) pointed out, gene duplication in fishes usually involves a doubling of the entire genome resulting in a tetraploid fish, but tetraploidy has been seen in only eight species of cyprinids, none of them shiners. The duplications of the gene are thought to be quickly fixed for a different allele (apparent heterozygosity) or silenced by the loss of the gene or by a mutation of a regulatory gene (rediploidization) (Ferris and Whitt, 1977a). Duplication of a single gene without polyploidy, though rare, has been seen for the PGI1 locus in the diploid loach

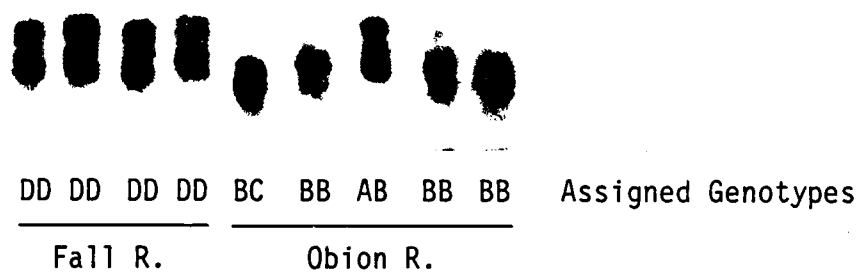


Figure 7. Comparison of stains at the MDH1 locus for two populations of Notropis camurus.

Acanthopsis choirorhynchus while a congener, A. semicinctus, did not show it for that locus (Ferris and Whitt, 1977b). Turner et al (1980) examined the four species of the genus Skiffa (family Cobitidae), which showed no evidence of ancestral tetraploidy. Three of the species had a single band at the MDH1 locus. The fourth species had two MDH1 bands and each individual appeared identical to the same heterozygous condition sometimes seen in its sister species. The authors attributed this to a relatively recent gene duplication at this locus. A similar situation on a different scale seems to present itself here, whereby some populations of a species exhibit duplication while others do not. Kuhl et al (1976) saw evidence for a single gene duplication in one specimen out of 20 examined of the characid species Cheirodon axelrodi for the PGI locus. If such a duplication is selectively advantageous, it is feasible that it could spread throughout a population, leading to a situation similar to that of Notropis camurus.

Since N. whipplei and N. galacturus also show the duplicated condition for MDH1, it would be safe to assume that this is the primitive state for N. camurus. This would necessitate the silencing of one of the copies of the gene in eastern populations. A pattern of gene duplication and differential rediploidization was described by Ferris and Whitt (1977a) for various species of suckers (Cotto-
stomidae), with the duplicated loci being considered in the primitive state. It is my hypothesis that the single polymorphic MDH1 band originated quite recently, after the splitting of N. camurus and

N. galacturus from a common ancestor, through gene silencing in the eastern populations of N. camurus in West Tennessee or northern Mississippi. An alternative is that the duplication is more recent and the single band is ancestral. If one accepts Gibbs (1957a, 1961) theory that N. galacturus originated in the Tennessee River system from an ancestor common to N. camurus, this would require independent duplication of the MDH1 locus for the two species, since they would have speciated from a single-banded ancestor. While the different mobilities of the N. galacturus bands as compared to those of N. camurus make this not unrealistic, the identical mobility of the N. whipplei double band to N. camurus renders an ancestral single band unlikely for the subgenus as a whole.

The fact that the southern Mississippi population of N. camurus in the Buffalo River exhibits a double band identical to the fish in Kansas needs consideration. One possible explanation is that the rediploidization to the north is of such recent origin that it has not had time enough to spread that far south. Another explanation, not necessarily exclusive of the first, is that gene flow from the west, coupled perhaps with selective pressures favoring the double band, have served to maintain that condition in southern Mississippi. Both explanations require consideration of dispersal via major river channels (Mississippi and Arkansas Rivers). The paucity of ichthyological collections from these habitats makes verification of this difficult at best. In any event, the Buffalo River population of

N. camurus shows, on the basis of the MDH1 locus, definite affinities to those in the Arkansas River system.

Such an explanation does not aid in scoring the locus for analysis of allele frequencies. As previously stated, having an all-heterozygote population violates the genetic bases of our investigation and is no doubt erroneous. On the other hand, an extra locus in a population complicates the calculation of genetic similarities between populations. For want of a better interpretation, I have somewhat arbitrarily assigned the double band a designation appropriate to a new allele at the same locus (D). Although this may not be the case, as no new allele is obvious, it hopefully neither exaggerates nor minimizes the genetic divergence between populations represented by a gene duplication. Both of the out-group species also displayed double-banded MDH1, but in N. galacturus the double band was at a different mobility than for N. camurus and N. whipplei. It was assigned a new allelic name as well (E). An alternative, also pursued, would be to eliminate MDH1 altogether from quantitative analysis.

A major difference between the two races of N. camurus is the lower levels of heterozygosity displayed by the western populations (Tables 6 and 7, pp. 19 and 20). These fish were fixed at 18-19 out of 20 loci per population at the .95 level. compared to 12-16 loci eastern populations south to the Big Black River. Even loci that were quite polymorphic in the east, such as EST, MDH1, and PEP2

were monomorphic in western populations. This means that certain alleles at those loci that appeared in the lower Mississippi race were absent from the Arkansas race. On the other hand, there were a few alleles (PEP1-A,C, ODH1-C) shown by the Arkansas race and not the lower Mississippi race but these all occurred at less than .05 frequency. The MDH1 locus is the only one that has any semblance to a fixed difference between any populations of N. camurus.

According to the similarity matrix (Table 6, p. 19) and phenogram (Figure 4, p. 21) inclusive of MDH1, each race of N. camurus forms a closely related cluster that is at least as divergent from the other race as it is from the closest related species, N. galacturus. The similarity values within and among species are comparable to those found in other cyprinids (Buth, 1979; Buth and Burr, 1978). Buth and Burr (1978) found in the genus Campostoma that a particular subspecies of C. anomalum was more similar to another species, C. oligolepis, than it was to another subspecies of C. anomalum. Such a situation presents itself here, where N. galacturus appears to have more genetic similarity to the western form of N. camurus than the eastern form does. The evidence for subspecific designation for the two forms of N. camurus is strong based on the phenogram.

The Buffalo River population from southern Mississippi presents an anomaly in the pattern of geographic variation. Although it resides east of the Mississippi River, it appears to be more closely related to the populations in the west than to the other populations

in the east (Figure 4, p. 21). It is identical in allele frequency to the west for one locus (MDH1), similar to the east for another (PEP2), and intermediate for yet another (EST). Its average heterozygosity is the lowest of the eastern populations, a tendency characteristic of the western form. It is interesting to note that the morphological characteristics measured (Figures 2 and 3, pp. 15 and 16, Table 4, p. 14) do not show a parallel pattern of variation. The southern Mississippi population, represented by the Homochitto River, was not closer to the western form than any other of the eastern populations for CPD, DO-LL, or LL scales. Though not quantified, the depigmented caudal base was very distinct in fish from the Homochitto and Buffalo Rivers, a characteristic more typical of fish from Kansas than from West Tennessee.

If MDH1 is excluded (Table 7, p. 20, Figure 5, p. 22), the situation is modified somewhat. Clearly, this locus is an important difference between the eastern and western forms. The major difference in clustering is the close affinity the Buffalo River population now shows to other eastern populations. In addition, overall similarities between east and west are increased, as well as similarity between N. galacturus and N. camurus. Regardless of the scoring method of the double band, the MDH1 locus has the only fixed difference between the two species.

If we consider both the morphological and the biochemical evidence, we seem to have a situation where different and varied

characters have different distribution patterns. The discordance between characters centers around the fish in southern Mississippi, so that each line of evidence examined independently will show this group to have closer affinity to one form or another or of intermediate nature. Due to the disjunct distribution of the species, patterns of gene flow are difficult to relate to phenetic relationships, although gene flow cannot be assumed to be nonexistent. The Buffalo River population is widely disjunct from the western populations, separated by most of the lower Arkansas River where N. camurus is replaced by N. whipplei and by a long stretch of the Mississippi River from the mouth of the Arkansas River to southern Mississippi (Figure 1, p. 2). To the north, however, its conspecifics are fairly close, separated by relatively few river miles of the Mississippi River, but these are the populations to which it shows the least amount of genetic similarity.

Clarification of the situation may be possible by biochemical analysis of N. camurus in the rivers between the Homochitto and the Big Black Rivers in Mississippi. Though few and relatively small drainages, these rivers such as Bayou Pierre, Coles Creek, and St. Catherine Creek are the boundary between the "typical eastern form found in the Big Black River and the "intermediate" form found in the Buffalo River. Particular attention should be paid to the MDH1 locus to see if the duplicated gene shows a clinal or a disjunct distribution. Since previous studies of duplicated genes in diploid

fishes involved separate species (Ferris and Whitt, 1977b; Turner et al, 1980) or single individuals in a population (Kuhl et al, 1976), reproductive compatibilities and inheritance for single versus double bands at the same locus is virtually unknown. A study at the population level could shed light on this problem. A possible indication of the nature of this aspect of duplicate genes may be seen in one individual of the Buffalo River population, whose slower band at the MDH1 locus appeared to be a heterozygote and resembled a BC genotype common in drainages to the north. The faster band of the duplicate gene for this individual was no different from other fish in the population. I suspect that it is the fast band that has become silenced and the slow that has become polymorphic farther north.

The EST locus may provide additional information. The eastern form has three fairly abundant alleles at this locus, while the western fish has but one of them, the slowest. The Buffalo River population lacked the fastest allele entirely and had the slowest at the highest frequency of any population east of the Mississippi River, a condition that could be interpreted as intermediate. Analysis of this locus may also show a cline whereby the eastern form gradually gives way to the western form as one moves south. For completeness sake, any study addressing this problem should also include the drainages still further south in Louisiana to show the whole extent of any cline that may exist.

Regarding any taxonomic decision, it is clear that the West Tennessee and northern Mississippi populations of N. camurus have

diverged from those in Kansas to the level of subspecies at least; however, the Buffalo River population makes such a designation difficult. In this instance, it would be wise to heed the note of caution expressed by Mayr (1969) about naming subspecies in cases where different characters show discordant patterns of variation. Morphological and ecological data place the Buffalo River fish with the eastern form of N. camurus. Biochemically, its affinities depend upon which similarity matrix is used, with or without MDH1. Buth (1984) maintains that more emphasis should be placed on the patterns of variation within a group than on the magnitude of variation. This does little to clarify the situation, for the allozymes indicate different affinities also, depending upon which one is focused on (PEP2, MDH1, or EST). Until further analysis is conducted to clarify the relationships of the southern Mississippi populations, the racial status of the two forms of N. camurus should remain.

The genetic relationships within the range of N. camurus defy any simple zoogeographic explanation. While Gibbs (1961) postulated a link between the two races in the northern part of the range with subsequent range contraction and disjunction, genetic similarities indicate a more recent link or avenue for gene flow in the southern part of the range. Allowing dispersal downstream via the Arkansas and Mississippi Rivers by the western form, it is difficult to explain why the Big Black River population then does not show more similarity to the western form, as the mouth of that river lies in closer proximity to the Arkansas River mouth than does the Buffalo River. Instead,

it is the most different from the western fish of all the eastern populations, after the Hatchie River fish. Zoogeographic interpretations are no doubt beyond the scope of this paper.

Finally, the questions concerning interspecific relationships of N. camurus are at least answerable with some degree of confidence. The electrophoretic evidence (Table 6, p. 19, Figure 4, p. 21) seem to support Gibbs (1957a, 1961) conclusion that N. galacturus is the closest related species to N. camurus. Even excluding MDH1 (Table 7, p. 20, Figure 5, p. 22), which was a fixed difference between the two species, N. galacturus is considerably more similar to N. camurus than is N. whipplei.

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LITERATURE CITED

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APPENDICES

APPENDIX A

University of Kansas

Neosho River system: #7887, 12215, 12231, plus two series
donated to The University of Tennessee, Knoxville.

Fall River system: #15690

Upper Arkansas River system: #16947

University of Michigan

Neosho River system: #123431, 142167

Homochitto River system: #155335, 155336, 161200, 161210

University of Tennessee

Obion River system: #44.337, 44.458, 44.461, 44.565, 44.654,
44.656, 44.684, 44.904, 44.1576, 44.2366

Forked Deer River system: #44.153, 44.1133, 44.1149, 44.1260,
44.1261, 44.1266, 44.1299, 44.1300, 44.1302, 44.2406

Hatchie River system: #44.286, 44.323, 44.532, 44.616, 44.838,
44.842, 44.850, 44.855, 44.857, 44.859, 44.860, 44.2438

Loosahatchie River system: #44.155, 44.1120, 44.1126

Yazoo River system: #44.884, 44.917, 44.921, 44.948, 44.2322,
44.2325, 44.2329

Big Black River system: #44.2697

Tennessee River system: #44.963, 44.1025, 44.1096, 44.1141,
44.1311

Neosho River system: #44.535, 44.3092 (all but one specimen
of this series provided by the University of Kansas)

Upper Arkansas River system: #44.3141 (this series also provided
by the University of Kansas)

APPENDIX B

Gibbs (1961) noted that the Arkansas race of Notropis camurus tended toward a higher count of pre-dorsal circumferential scales above the lateral line. These counts were also taken, according to Hubbs and Lagler (1947), and are given in Table B-1. They generally agree with Gibbs' (1961) counts and divergence between races is not great enough to be a factor in considerations discussed in the text.

Measurements were also taken of caudal peduncle length according to Hubbs and Lagler (1947) to see if there was any difference in lengthwise body proportions between the two races. Expressed as percent standard length, these values are given in Table B-2 below for each size class of fish. Despite a slight tendency for the Arkansas race toward a longer caudal peduncle, the variation within populations and size classes was too great to allow any credible conclusions to be drawn from this data. Figure B-1 is a graphic representation of the mean and variation of this measurement for each population.

Table B-1. Frequency Distribution of Predorsal Circumferential Scales Above the Lateral-Line for Notropis camurus

River system	Pre-dorsal circumferential scales above lateral line					N
	11	12	13	14	15	
<u>East</u>						
Tennessee		3	- 7	1		11
Obion		15	123	15	1	154
Forked Deer	1	8	76	10		95
Hatchie	6	35	188	8	1	238
Loosahatchie		3	29	3		35
Yazoo/Big Black	1	6	100	19	10	136
Homochitte	10	44	147	3		204
<u>West</u>						
Fall			19	3	1	23
Neosho			110	29	2	141
Upper Arkansas		1	6	2		9
East total	18	114	670	59	12	873
West total		1	135	34	3	173

Table B-2. Frequency Distribution of Caudal Peduncle Length for Notropis camurus, Expressed as Percent Standard Length

River system	mm SL	Caudal peduncle length/standard length									
		17	18	19	20	21	22	23	24	25	
Tennessee	70+					6	1				
	60-69.5			1		2		1			
Obion	70+		1	2	8	9	6	8	1		
	60-69.5	1		1	7	13	2	3			
	55-59.5				2	7	6	1			
	50-54.5				3	8	11	1	2		
Forked Deer	70+			2	7	3	12	2			
	60-69.5				2	5	6	4		1	
	55-59.5				1	3	1				
	50-54.5				3	2	3	2			
Hatchie	70+		1	2	9	23	15	13	1		
	60-69.5				10	21	17	7	2	1	
	55-59.5			3	4	14	11	5	3	1	
	50-54.5			2	10	24	9	5	1		
Loosahatchie	70+				1	5	5	5			
	60-69.5				2	1	8	3	2		
Yazoo/Big Black					3	2	8				
	60-69.5		1	1	6	2	4	2			
	55-59.5			1	4	6	3	2			
	50-54.5			1	4	4	10	3			
Homochitto	70+					4	2	2			
	60-69.5				1	4	5	3			
	55-59.5			4	6	5	5	2			
	50-54.5			2	7	15	10	5	1		
Fall	60-69.5						1	1	1	2	
	55-59.4						1				
	50-54.5						1		1		

Table B-2 (Continued)

River system	mm SL	Caudal peduncle length/standard length								
		17	18	19	20	21	22	23	24	25
Upper Arkansas	70+						1	1	1	
	60-69.5		1		2					
	50-54.5			1	1					
Neosho	70+					5	4	5	3	
	60-69.5				2	7	4	1	2	
	55-59.5				3		5	4		
	50-54.5			1	1	3	1	4	1	

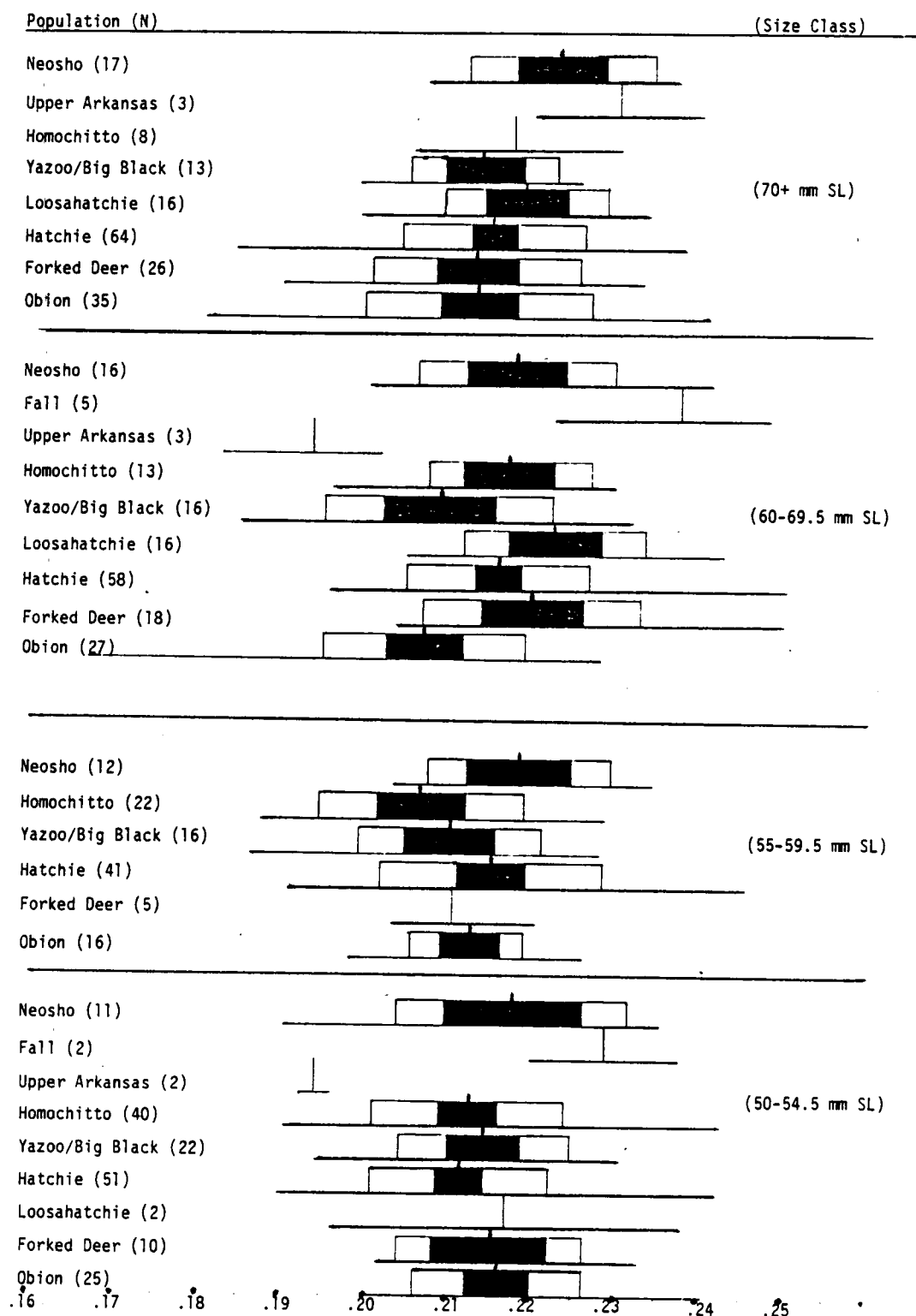


Figure B-1. Graphic analysis of caudal peduncle length for *Notropis camurus*, expressed as percent standard length. Horizontal line=range; vertical line=mean; open rectangle=one standard deviation on each side of mean; black rectangle=two standard errors on each side of mean (Hubbs and Hubbs, 1953).

APPENDIX C

Tissue Processing

At processing, all fish were gutted. Larger fish were filleted, removing 2g or less of muscle tissue. Smaller fish were beheaded and betailed and the entire remainder used. The fillet or body was then weighed to the nearest .1 g and subsequently chopped with a razor or scissors until it was a fine mush. Each fish was placed in a separate vial and grinding buffer pH 6.8 was added, the number of milliliters grinding buffer being equivalent to the weight of the tissue in grams. After thorough mixing, the tissue was refrigerated in the buffer solution for at least 40 minutes. After centrifugation for 40 minutes at 13,500 rpm, the supernatant was pipetted out and placed in test tubes. The test tubes were then put in the deep-freeze until the gels were run, a period not exceeding 3 months.

Gel Preparation

Gel preparation and loading was modified from Selander et al (1971). The 47 g of starch used per 400 ml gel buffer was made up of 10 g of Sigma-starch and 37 g of Electrostarch for the LiOH buffer and 17 g Sigma-starch and 30 g Electrostarch for the other buffer systems. The starch solution was brought to a boil over a Bunsen burner with constant swirling. After boiling 30-40 seconds, the solution was immediately subjected to a vacuum by aspiration for 50 seconds after which it was poured into the lucite molds. The

gels were allowed to harden overnight before use, being wrapped in plastic to avoid drying out.

The LiOH and Poulik gel buffers and the Borate electrode buffer were prepared and used according to Selander et al (1971) except that the LiOH gel was run at 325 v. The Histidine gel buffer and its Na-citrate electrode buffer were prepared according to Brewer (1970). The tris-EDTA borate (TEB) gel buffer was prepared by dissolving 30.25 g tris, 3.0 g EDTA, and 2.3 g boric acid in 1 liter of $d-H_2O$, pH 8.8. For the gel, 133 ml buffer solution was diluted to 400 ml with distilled water. Borate electrode buffer (Selander et al, 1971) was used with TEB and the gel was run for 4 hours at 200 v.

Stain Recipes

Creatine kinase (CK), esterase (EST), glucose-6-phosphate dehydrogenase (G-6-PD), mannose-6-phosphate isomerase (MPI), and peptidase (PEP) recipes were modified from Harris and Hopkinson (1978), Lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucose isomerase (PGI), and phosphoglucomutase (PGM) recipes were modified from Selander et al (1971). All stains were incubated in the dark at 37°C unless otherwise indicated. For most recipes, p-nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), 4,5-dimethylthiazolyl-2 (MTT), nicotinamide adenine dinucleotide (NAD), and nicotinamide adenine dinucleotide phosphate (NADP) were given in solid amounts. For our purposes, the volume of a solution is given. For each but MTT, 1 g was dissolved in 100 ml of water for the solution. For MTT, it was 1 g per 125 ml water. Changes in recipes are given below.

CK: Stain-25 ml .2 M tris-HCl, 25 mg ADP, 900 mg glucose, .05 mg hexokinase, 50 mg phosphocreatine, 1.1 ml .1 M $MgCl_2$. With this stain, an agar overlay method was used. Agar-500 mg ionoagar added to 25 ml water, brought to a boil, and allowed to cool to 70°C. Stain mixture then added, followed by 1.75 ml NADP, .6 ml PMS, and .6 ml MTT. It was then quickly poured over the gel and allowed to harden in the incubator.

EST: 50 ml Na-phosphate buffer pH 7.0. For esterase, eserine sulfate (ES) was used as an inhibitor. 33 mg ES was put in the Na-phosphate buffer and poured over the gel. After sitting under lights for 10 minutes, the Na-phosphate was poured off into a beaker, the salt (FBRR) and substrate (alpha-naphthyl acetate) were added, and poured back on the gel and placed in the incubator.

G-6-PD: 47 ml .05 M tris-HCl pH 8.0, 3 ml .1 M $MgCl_2$. MTT was not used, but .5 ml NADP, .5 ml NBT, and .2 ml PMS were.

MPI: An agar method was not used. 45 ml tris-HCl pH 8.0, 1.2 ml G-6-PDH, .5 ml PGI, 2 ml .1 M $MgCl_2$, 2.0 ml NADP, .2 ml PMS, and 2.0 ml MTT.

PEP: Na_2HPO_4 , 3-amino-9 ethyl carbazole, and agar were not used. 20 ml L-phenylalanyl-L-leucine, 10 mg O-dianisidine, 50 ml .2 M tris-HCl pH 8.0, 20 mg peroxidase, 10 mg snake venom, .25 M $MnCl_2$.

LDH: No water was used. 40 ml tris-HCl, 1.3 ml NAD, .3 ml NBT, .5 ml PMS.

MDH: 40 ml tris-HCl, 6 ml D-L malic acid, 2.5 ml NAD, 2.5 ml NBT, .6 ml PMS.

PGI: Stain-15 ml H₂O, 10 mg fructose-6-phosphate, .3 ml .1 M MgCl₂, .3 ml G-6-PDH, .6 ml NADP, .1 ml PMS, 1.2 ml MTT. An agar overlay method similar to the one given for CK above was used except that the ionoagar was dissolved in 25 ml .2 M tris-HCl pH 8.0.

PGM: 70 mg glucose-1-phosphate, .5 ml NADP, .5 ml PMS, 1 ml MTT. For economic reasons, the glucose-1,6-diphosphate was not used and the amount of water was increased 5 ml to 30 ml. Results were identical, although staining took a bit longer.

In addition, two other stains were used, octanol dehydrogenase-indophenol oxidase (ODH/IPO) and general protein (GP).

ODH/SOD: 1 ml primary octanol added to 50 ml .2 M tris-HCl pH 9.0. After adding 5 ml NAD, 1.3 ml NBT, and .1 ml PMS, the gel was incubated at room temperature under lights.

GP: 2 g buffalo black (=naphthol blue black) in 100 ml fixative (5:5:1 of methanol:water:acetic acid). Cover gel with stain, let stand for 1.5 hours, then pour off. Add pure fixative to leach out color. Fixative must be changed every .5-1 hour several times. Let leach overnight.

The following are localities for fish collected for electrophoresis:

Obion system-N. Fork Obion River, 1.8 air mi. NW of Jones Mill at Tn-69. Henry Co., Tenn.

Forked Deer system-Middle Fork Forked Deer River, .25 mi.
S of I-40 at Co. Rd. 8039, Henderson Co., Tenn.

Hatchie system-Clover Creek at Tn-18, Hardemann Co., Tenn.

Loosahatchie system-Loosahatchie River at Co. Rd. 8078.
Hardemann Co., Tenn. (32 spec.). Scotts Creek at old Brownsville
Rd., 1.0 mi. NW of Jct. with Canada Rd., Shelby Co., Tenn. (5 spec.)
(collected by Andrew Haines).

Big Black system-unnamed creek, second creek S of Grenada
Co. line along Hwy. 51, NW of Duck Hill. Montgomery Co., Miss.

Buffalo system-Buffalo River at unnamed rd. NE of Centerville,
Wilkinson Co., Miss. (collected by Robert Cashner).

Upper Arkansas system-Chikaskia River, Sumner Co., Kansas
Sec. 36, T335 R3W (collected by Don Distler et al).

Neosho system-Shoal Creek at Ks-26 Cherokee Co., Kansas (3
spec.), Cottonwood River, just N of Cottonwood Falls at Ks-177, Chase
Co., Kansas (30 spec.).

Fall system-Fall River at Ks-96, S of Fredonia, Wilson Co.,
Kansas (4 spec.), Fall River at Co. Rd. xing 5 mi. W of Fredonia,
Wilson Co., Kansas (29 spec.).

Notropis galacturus-Citico Creek at creek km 16.4, Monroe
Co., Tenn.

Notropis whipplei-Clinch River at Frost Ford, Hancock Co.,
Tenn.

APPENDIX D

Hillis (1984) took issue with the widespread use of Nei's (1972) identity values, stating that its basic assumption of equal rates of mutation for all genes is rarely, if ever, met. Hillis' modified value is an average of identity values calculated between populations for each locus. The matrix of genetic similarity and distance is given in Table D-1 and the derived phenogram using UPGMA method is shown in Figure D-1. The similarity values were changed somewhat, mostly decreasing them in cases where one of the populations had a higher degree of heterozygosity than the other. Relative relationships between populations remained unaltered in comparison to the analysis given in the text.

Table D-1. Matrix of Hillis' (1984) Genetic Similarity (above diagonal) and Distance (below diagonal) Values Between Populations Examined in This Study

Population	1	2	3	4	5	6	7	8	9	10	11
1. Obion N.c.		.991	.973	.975	.962	.937	.914	.915	.913	.940	.793
2. Forked Deer N.c.	.009		.976	.981	.960	.932	.901	.902	.900	.932	.779
3. Hatchie N.c.	.028	.024		.996	.953	.901	.873	.873	.872	.895	.737
4. Loosahatchie N.c.	.025	.020	.004		.959	.912	.889	.889	.888	.903	.741
5. Big Black N.c.	.039	.041	.048	.042		.911	.885	.885	.883	.905	.761
6. Buffalo N.c.	.065	.071	.104	.092	.093		.987	.987	.985	.943	.836
7. Upper Arkansas N.c.	.090	.104	.136	.118	.123	.014		.999	.998	.926	.811
8. Neosho N.c.	.089	.103	.136	.118	.122	.013	.001		.999	.926	.811
9. Fall N.c.	.091	.106	.137	.119	.124	.015	.002	.001		.925	.811
10. N.g.	.062	.071	.110	.102	.099	.059	.077	.077	.078		.796
11. N.w.	.231	.250	.305	.299	.273	.179	.209	.209	.210	.228	

Note: N.c.--N. camurus; N.g.--N. galacturus; N.w.--N. whipplei.

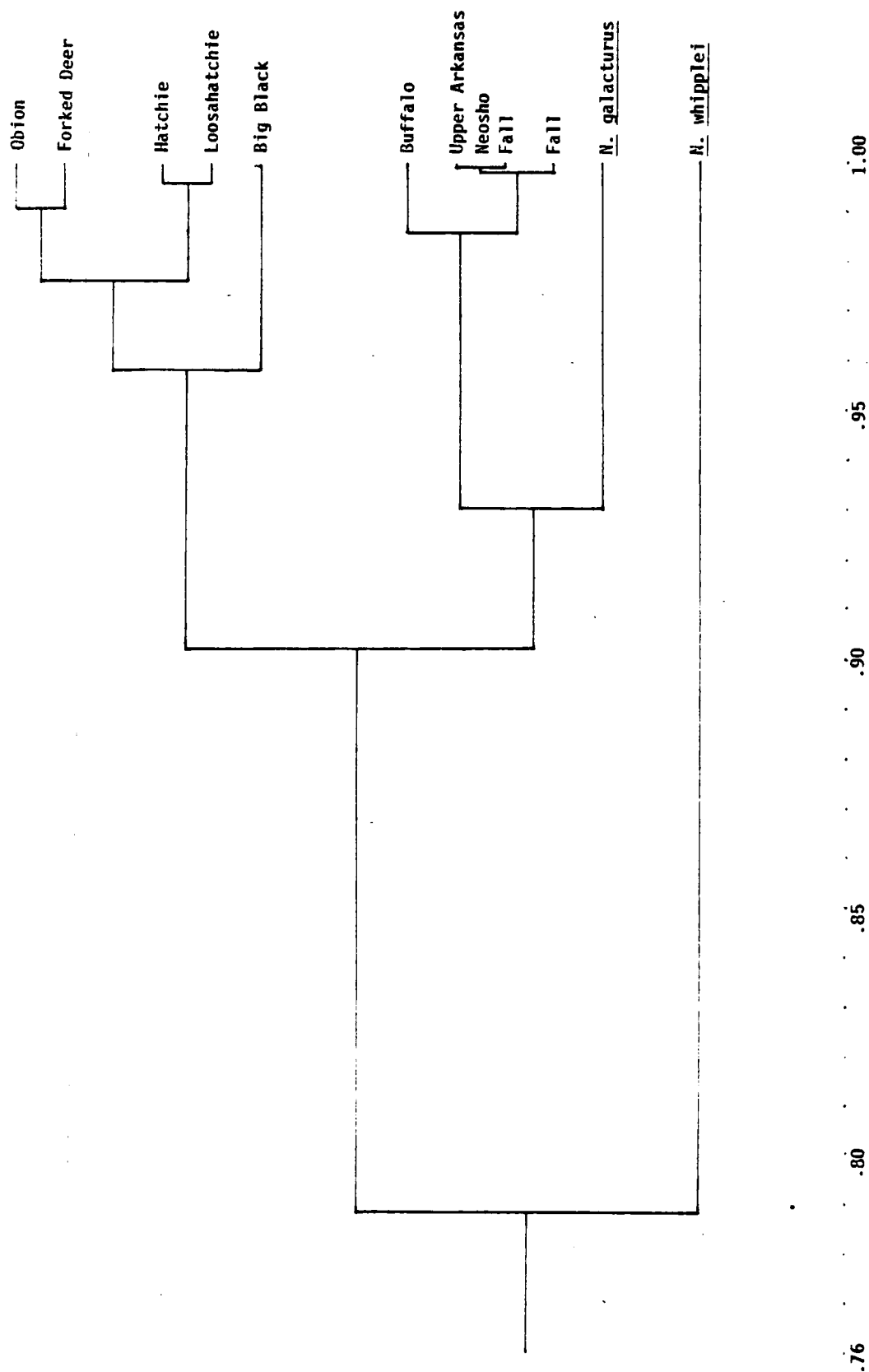


Figure D-1. Phenogram of genetic similarity calculated using Hillis' (1984) modified identity values and UPGMA over 20 loci. All populations are of Notropis camurus unless otherwise indicated.

VITA

Richard Gene LeDuc was born under the sign of Aquarius on February 11, 1958, in Santa Monica, California. He attended elementary school in Woodland Hills, California, and graduated from Crespi High School in Encino in June 1975. He received an Associate of Arts degree from Pierce Community College in June 1977 and his Bachelor of Arts Degree in Biology in June 1980 from California State University Northridge. He accepted a teaching assistantship and entered The University of Tennessee, Knoxville, in the fall of 1981 and began striving toward a Master's degree. This goal was achieved in December 1984.

The author is not a member of anything and looks forward to an early retirement.