



Biochemical Genetics of Sunfish IV. Relationships of Centrarchid Genera

Author(s): John C. Avise, Donald O. Straney, Michael H. Smith

Source: *Copeia*, Vol. 1977, No. 2 (May 25, 1977), pp. 250-258

Published by: American Society of Ichthyologists and Herpetologists

Stable URL: <http://www.jstor.org/stable/1443906>

Accessed: 25/11/2008 14:40

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/page/info/about/policies/terms.jsp>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/action/showPublisher?publisherCode=asih>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is a not-for-profit organization founded in 1995 to build trusted digital archives for scholarship. We work with the scholarly community to preserve their work and the materials they rely upon, and to build a common research platform that promotes the discovery and use of these resources. For more information about JSTOR, please contact support@jstor.org.



American Society of Ichthyologists and Herpetologists is collaborating with JSTOR to digitize, preserve and extend access to *Copeia*.

<http://www.jstor.org>

Biochemical Genetics of Sunfish IV. Relationships of Centrarchid Genera

JOHN C. AVISE, DONALD O. STRANEY AND MICHAEL H. SMITH

We have examined electrophoretic variation in proteins encoded by 11-14 loci in species representing all nine genera of the Centrarchidae. A dendrogram based on allozyme information is compared to postulated relationships of sunfish genera based on general and specific morphologies, and on hybridizing propensity. The allozyme information correlates most strongly with that derived from a very detailed study of the acoustico-lateralis system by Branson and Moore (1962). Similarities between the two sets of data are observed 1) in the clustering together of species of *Lepomis*; 2) in the clustering of *Lepomis* with *Micropterus*; 3) in the placement of *Acantharchus* with *Archoplites*; and 4) in the very distant relationship of *Elassoma* to the other centrarchid genera. The levels of genetic similarity between centrarchid genera are compared to previously published levels of similarity between congeneric species of *Lepomis*, subspecies of *Lepomis macrochirus*, and geographic populations within the subspecies of *L. m. macrochirus* and *L. m. purpurescens*. Mean levels of genetic similarity (\bar{S}) are as follows: between genera, $\bar{S} = 0.29$; between congeneric species, $\bar{S} = 0.53$; between subspecies of *Lepomis macrochirus*, $\bar{S} = 0.85$; between geographic populations, $\bar{S} = 0.97$.

THE Centrarchidae is a dominant component of the North American ichthyofauna. About 30 living species belonging to nine genera are currently recognized. The earliest known fossils referable to Centrarchidae have been found in Eocene deposits (Romer, 1966), and some fossils assigned to living centrarchid genera may be as old as the Miocene (Miller, 1965). Centrarchids are indigenous to North America and probably evolved from serranid (sea bass) ancestors early in the Cenozoic.

Attempts to determine phylogenetic relationships among genera of sunfish have been based on morphological counts and measurements (Schlaikjer, 1937; Bailey, 1938), the acoustico-lateralis system (Branson and Moore, 1962), success of hybridization (Hester, 1970; West, 1970) and chromosome cytology (Roberts, 1964). As discussed later, results of these studies are far from uniform. Nonetheless, a general picture of the possible relationships among centrarchid genera has emerged, and is reflected in the current classification:

Centrarchidae	
Centrarchinae	Lepominae
Ambloplitini	Enneacanthini
<i>Archoplites</i>	<i>Enneacanthus</i>
<i>Ambloplites</i>	Lepomini
<i>Acantharchus</i>	<i>Lepomis</i>
Centrarchini	Micropterini
<i>Pomoxis</i>	<i>Micropterus</i>
<i>Centrarchus</i>	

This arrangement is from Childers (1967). *Elassoma* is not included; most authors agree that it is only distantly related to Centrarchidae, and perhaps should be placed in a distinct family (Branson and Moore, 1962).

In this study, we continue our survey of genetic variability and differentiation among members of Centrarchidae. Previous work has described geographic variation in *Lepomis macrochirus* (Avisé and Smith, 1974a), and differentiation among species of *Lepomis*, the most species-rich of centrarchid genera (Avisé and Smith, 1974b). Here we examine electrophoretically levels of genetic differentiation among species representing all nine sunfish genera. Results will be compared 1) to results of previous morphological and hybridization studies, and 2) to levels of genic differentiation between congeneric species, and between conspecific populations.

MATERIALS AND METHODS

Fish were frozen at time of capture and transported to the laboratory where routine procedures of horizontal starch gel electrophoresis were employed to assay variation in specific proteins. Electrophoretic techniques are those of Selander et al. (1971) as modified for fish by Avisé and Smith (1974a,b). The common allele at each locus in the bluegill (*Lepomis macrochirus*) was arbitrarily assigned a value of 100.

TABLE 1. SAMPLES OF SPECIES BELONGING TO NINE GENERA OF NORTH AMERICAN CENTRARCHIDAE.

Sample	Genus	Species	Common name	Collection site ¹	# individuals	Mean heterozygosities
1	<i>Lepomis</i>	(10 spp.)	sunfish	See Avise & Smith (1974b)	1099	5.9
2	<i>Pomoxis</i>	<i>nigromaculatus</i>	black crappie	Pond near Ellenton, S.C.	18	1.0
3	<i>Enneacanthus</i>	<i>obesus</i>	banded sunfish	Creek near Ellenton, S.C.	27	0.0
4	<i>Micropterus</i>	<i>salmoides</i>	largemouth bass	Pond near Ellenton, S.C.	18	8.3
5	<i>Elassoma</i>	<i>okefenokee</i>	pygmy sunfish	Baker Co., Fla.	20	1.4
6	<i>Elassoma</i>	<i>evergladei</i>	pygmy sunfish	Baker Co., Fla.	12	0.0
7	<i>Centrarchus</i>	<i>macropterus</i>	flier	Swamp near Ellenton, S.C.	4	0.0
8	<i>Acantharchus</i>	<i>pomotis</i>	mud sunfish	River Styx, Fla.	5	2.9
9	<i>Ambloplites</i>	<i>rupestris</i>	rockbass	Central Tennessee	12	13.0
10	<i>Archoplites</i>	<i>interruptus</i>	Sacramento perch	Bishop Lake, Calif.	39	0.4

¹ More detailed information available on request.

Other alleles were designated according to proportional mobility when run along side the 100 allele and beside other alleles of known relative mobility.

The proteins examined in this study were chosen solely on the basis of availability of suitable staining procedures and clarity of banding. Additional proteins banded well in some species but not in others. We have included in this paper only those loci which could be reliably scored in most or all species of Centrarchidae. The set of loci examined was chosen without bias with respect to level of differentiation between species.

Heterozygosities (mean percentages of individuals heterozygous per locus) were determined from allele frequencies using Hardy-Weinberg probabilities. Genetic similarities (S) between species were calculated according to Rogers' (1972) formula. Rogers' similarity coefficient may assume values from zero to one, with one indicating genetic identity. Genetic distance is $D = 1 - S$. From the genetic similarity matrix, cluster analysis was performed using the unweighted pair-group method with arithmetic means (Sokal and Sneath, 1963).

The species examined for the first time in this study are listed in Table 1. We have sampled one representative species from each of seven genera, two species of *Elassoma* and ten species of *Lepomis*. The genetic relationships among *Lepomis* have previously been described (Avise and Smith, 1974b). In order to avoid unnecessary repetition, mean values for these *Lepomis* species are used here wherever possible and where they do not distort the intended meaning. For example, since all *Lepomis* species cluster together in a biochemical dendrogram, distinct

from members of all other genera, we have simply included in the dendrogram the single category "*Lepomis*"; the point at which this category joins the remainder of the dendrogram is determined by the mean level of genetic affinity of all *Lepomis* species to the other genera.

A question arises whether the species sampled adequately represent each of the sunfish genera. The most species-rich genus *Lepomis* is well represented by 10 of its 11 species. *Acantharchus*, *Centrarchus* and *Archoplites* are monotypic genera, and *Pomoxis* and *Ambloplites* each contain only two living species. We have sampled one of three species of *Enneacanthus*, and two of the three species of *Elassoma*. We have sampled only one of six living *Micropterus* species, although a large-scale study of other species is planned. Since the possibility exists that *Micropterus salmoides* does not adequately represent all *Micropterus* species for purposes of intergeneric comparisons, it is best to consider the relationships of *Micropterus* presented in this paper to strictly apply only to *Micropterus salmoides*. Similar reservations apply to the other genera not exhaustively sampled.

We also admit that the number of loci which could be reliably scored in most or all species is regrettably small. To our knowledge, the 19 species studied here represent the largest number of species of any taxon surveyed electrophoretically (at a number of loci) to date. As more species are added to a survey, the difficulty of reliably scoring a large number of loci increases substantially. Because we have scored relatively few loci, we prefer not to advocate changes in the current taxonomy of centrarchid genera based on the new biochemical data.

TABLE 2. COMMON ALLELES (FREQUENCY ≥ 0.05) AT 14 LOCI IN SPECIES OF CENTRARCHIDAE.¹

Species	Locus													
	Ldh-1	Ldh-2	Idh-1	6Pgd-1	Es-1	Es-3	Mdh-1	Cot-1	Cot-2	Ipo-1	Fgm-1	Fgi-1	Fgi-2	Pep-2
<i>Lepomis humilis</i>	100	100	A	100	96	93	100	100(0.71)	100	122	110	96	137(0.87)	123
<i>Lepomis microlophus</i>	100	100	A	87	96	81	102	100	109	122(0.58)	110	90(0.70)	103(0.13)	123
<i>Lepomis auritus</i>	100	100	C	100	96	86(0.61)	102	100	58	96(0.42)	110	81(0.30)	100	123
<i>Lepomis gulosus</i>	100	100	A	100	100	63	100	100	58	110(0.91)	110	95(0.80)	62(0.33)	83(0.94)
<i>Lepomis megalotis</i>	100	100	A	87	100(0.37)	100(0.32)	102	114(0.07)	58	100	115(0.08)	89(0.19)	100(0.67)	88(0.06)
<i>Lepomis cyanellus</i>	100	100	A	88(0.50)	80	80	102	100	58	100(0.70)	110	98(0.86)	130	83
<i>Lepomis punctatus</i>	100	100	A	100	96	88(0.52)	102	100	58	122	110(0.30)	95(0.14)	130	83
<i>Lepomis gibbosus</i>	100	100	A	100	96	86(0.48)	102	100	58	122	110(0.78)	95(0.83)	100	88
<i>Lepomis marginatus</i>	100	100	A	87	100	87	102	65(0.78)	58	100	93(0.22)	90(0.17)	100	115
<i>Lepomis macrochirus</i>	100	100	A	100	100	94(0.46)	102	82(0.22)	58	100	110	90(0.35)	100	95
<i>Pomoxis nigromaculatus</i>	150	100	D	100	—	83(0.39)	102	100	70	74	110	95	100	85
<i>Enneacanthus obesus</i>	200	100	E	100	—	88(0.15)	102	100	100	—	125	92(0.06)	137	124
<i>Micropterus salmoides</i>	100	100	E	100	—	100(0.50)	102	100	58	—	115	102	140	90

TABLE 2. (Continued)

Species	Locus													
	Ldh-1	Ldh-2	Idh-1	6Pgd-1	Es-1	Es-3	Mdh-1	Got-1	Got-2	Ipo-1	Pgm-1	Pgi-1	Pgi-2	Pep-2
<i>Elassoma okefenokee</i>	102	100	A	82	—	—	98	65	100	—	105	60	150(0.82)	137(0.18) 30
<i>Elassoma evergladei</i>	102	100	A	82	—	—	98	65	100	—	105	81	198	30
<i>Centrarchus macropterus</i>	152	100	B	80	—	—	102	114	100	—	115	102	150	115
<i>Acantharchus pomotis</i>	160	100	A	100	—	—	100	140	100	—	115	100	170(0.80)	145(0.20) 49
<i>Ambloplites rupestris</i>	100	100	E	82(0.54) 100(0.46)	—	—	102	107	60(0.54) 100(0.46)	—	115(0.83) 120(0.17)	102	145(0.92) 142(0.08)	90
<i>Archoplites interruptus</i>	150	100	A	100	—	—	102	125	100	—	118	97	140	83

¹Allele (frequency) is given. The phenotypes A, B, and C listed for Idh-1 are pictured in Fig. 1. Not all populations were scored for Es-1, Es-3 and Ipo-1. The sample of *L. macrochirus* was taken from Enid Reservoir, Mississippi, and the allele 110 for Pgm-1 was formerly designated 112. (Avisé and Smith, 1974a).

Nonetheless, as described below, the results from the allozyme survey do agree well with classical systematic information, in broad perspective. In addition, they provide quantitative estimates of the proportion of genetic loci which typically differ in allelic composition between centrarchid genera.

RESULTS

Proteins encoded by a total of 14 genetic loci were scored in all species of *Lepomis*. Eleven loci were scored in each of the other species. Common alleles at these loci are listed in Table 2. The proteins examined represent a wide array of functional types, including dehydrogenases, oxidases, esterases, transaminases, mutases, isomerases, and peptidases. The proteins scored and the abbreviations of the loci which encode them are as follows: lactate dehydrogenases (Ldh-1 and Ldh-2); isocitrate dehydrogenase (Idh-1); 6-phosphogluconate dehydrogenase (6-Pgd-1); esterases (Es-1 and Es-3); malate dehydrogenase (Mdh-1); glutamate oxalate transaminases (Got-1 and Got-2); indophenol oxidase (Ipo-1); phosphoglucomutase (Pgm-1); phosphoglucose isomerases (Pgi-1 and Pgi-2); peptidase (Pep-2).

Most of these enzymes exhibit banding patterns on gels which are similar to those observed in other fishes where the genetic basis has been documented through breeding studies (May, 1975; Allendorf, 1975). In addition, the codominant inheritance of alleles at eight loci (Es-3, Mdh-1, Got-2, Ipo-1, Pgm-1, Pgi-1, 6Pgd-1 and Pep-2) has been confirmed by the appearance of appropriate banding patterns in F₁ hybrids between *Lepomis macrochirus* and *L. microlophus* (Avisé and Smith, 1974b). At other polymorphic loci where sample sizes were adequate to permit tests, observed genotype frequencies were close to those predicted on the basis on Hardy-Weinberg equilibrium, further increasing confidence in our correct interpretation of zymogram patterns.

One enzyme, isocitrate dehydrogenase, did exhibit unusual banding patterns in several species. Individuals of most species uniformly exhibited a single band, presumably representing the product of alleles at a single monomorphic locus. However, five species (*Lepomis auritus*, *Pomoxis nigromaculatus*, *Ennaecanthus obesus*, *Micropterus salmoides* and *Ambloplites rupestris*) showed various 2-banded phenotypes, both bands equally intense (Fig. 1). All members within a given species had identical patterns, with no apparent variants. Isocitrate dehydro-

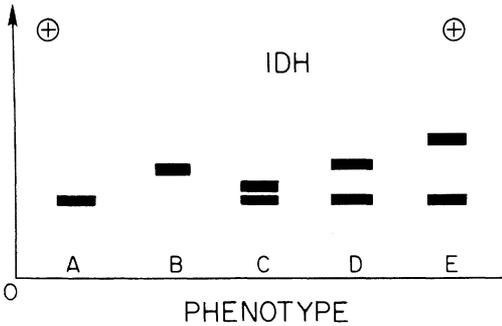


Fig. 1. Zymogram patterns of isocitrate dehydrogenase in 19 species of North American Centrarchidae. Most species exhibited either phenotype A or B. Phenotype C was observed in *Lepomis auritus*; phenotype D in *Pomoxis nigromaculatus*; and phenotype E in *Enneacanthus obesus*, *Micropterus salmoides* and *Ambloplites rupestris*.

genase is a dimeric molecule in most animals, including fishes. If two loci (perhaps arising through a gene duplication) are involved in determining the two banded zymogram in these five species, the products of the two loci do not form a heterodimer band of expected intermediate mobility. The lack of active hybrid enzyme molecules between products of different *Idh* loci has however been reported in fishes of the genus *Xiphophorus* (Siciliano, 1973). Since we cannot unambiguously determine the exact number of *Idh* loci in sunfishes with the present data, and since the zymograms do indicate at least some genetic differences between *Idh* in different species, for purposes of interspecies comparisons we have conservatively scored each distinct *Idh* phenotype (Fig. 1) as the product of a different allele at a single genetic locus.

The level of genic heterozygosity at 11 loci in general species of sunfish (Table 1) is considerably lower than the mean of about 6% reported for all vertebrates examined (Selander and Kaufman, 1973). No variability was observed in *Enneacanthus obesus*, *Elassoma evergladei* or *Centrarchus macropterus*. However, these results should be interpreted with extreme caution. First, only small samples were obtained of some species such as *Centrarchus*. More importantly, the number of loci examined is not large, and some classes of enzymes which are known to be particularly variable could not be reliably scored in all genera of Centrarchidae, and hence are not included in the heterozygosity estimates. Among *Lepomis* species, for which 14 loci including two esterases were scored, mean heterozygosity was 5.9%.

Allele frequencies were used to calculate measures of relatedness (genetic similarity and genetic distance) between each pair of the 19 sunfish examined (Table 3). Genic similarities between members of different genera cover a broad spectrum, ranging from a low of 0.09 in the comparison between *Pomoxis nigromaculatus* and *Elassoma evergladei* to a high of 0.53 in the comparison between *Enneacanthus obesus* and *Ambloplites rupestris*. Mean level of genetic similarity (Rogers' coefficient) between all 36 possible pairwise intergeneric comparisons equals 0.29. Genic similarities between genera of centrarchids have not previously been reported. However, electrophoretic data have been collected for a few species, primarily by Whitt and associates. Probably the most complete set of data includes information on 14 genetic loci in *Micropterus salmoides* and *Lepomis cyanellus* (Whitt et al., 1973). Using their data, we have calculated genetic similarity using Rogers' coefficient; it equals 0.43. Based on our own data, genetic similarity between these two species equals 0.42.

An expanded version of the matrix of genetic similarities in Table 3 was employed to form a dendrogram by cluster analysis (Fig. 2). The estimates of the genic relationships among centrarchid genera may be compared to previously inferred relationships based on other criteria. In order to make these comparisons as objective as possible, several procedures have been used. First, we have arbitrarily assigned similarity values to the various levels of the "formal classification" of Centrarchidae as presented in the introduction. The assigned similarity values were chosen to cover roughly the same range of values as had been obtained from the genic evidence—that is, from about 0.10 to 0.55. The simplest and most reasonable method of assignment is to equally divide the range of values to be assumed by the number of recognized levels of the taxonomic hierarchy. Assignments were made as follows: 1) species belonging to the same tribe, 0.55; 2) species belonging to different tribes in the same subfamily, 0.40; 3) species belonging to different subfamilies, 0.25; 4) species of *Elassoma* versus other centrarchids, 0.10. These values are listed below the diagonal in Table 3.

The most thorough published study of centrarchid phylogeny is based upon a very detailed analysis of components of the acoustico-lateralis system (Branson and Moore, 1962). From their hypothetical dendrogram, we have constructed a dendrogram from which similarity estimates

TABLE 3. LEVELS OF SIMILARITY BETWEEN CENTRARCHID GENERA.¹

	1	2	3	4	5	6	7	8	9	10
1. <i>Lepomis</i> (mean of 10 species)		0.32 (0.68)	0.30 (0.70)	0.45 (0.55)	0.21 (0.79)	0.22 (0.78)	0.18 (0.82)	0.30 (0.70)	0.31 (0.69)	0.33 (0.67)
2. <i>Pomoxis nigromaculatus</i>	0.25 (0.25)		0.37 (0.63)	0.36 (0.64)	0.11 (0.89)	0.09 (0.91)	0.18 (0.82)	0.19 (0.81)	0.25 (0.75)	0.37 (0.63)
3. <i>Enneacanthus obesus</i>	0.40 (0.35)	0.25 (0.25)		0.36 (0.64)	0.19 (0.81)	0.18 (0.82)	0.45 (0.55)	0.37 (0.63)	0.53 (0.47)	0.46 (0.54)
4. <i>Micropterus salmoides</i>	0.40 (0.40)	0.25 (0.25)	0.40 (0.35)		0.12 (0.88)	0.11 (0.89)	0.21 (0.79)	0.30 (0.70)	0.40 (0.60)	0.34 (0.66)
5. <i>Elassoma okefenokee</i>	0.10 (0.10)	0.10 (0.10)	0.10 (0.10)	0.10 (0.10)		0.83 (0.17)	0.26 (0.74)	0.29 (0.71)	0.20 (0.80)	0.28 (0.72)
6. <i>Elassoma evergladei</i>	0.10 (0.10)	0.10 (0.10)	0.10 (0.10)	0.10 (0.10)	0.70 (0.70)		0.18 (0.82)	0.28 (0.72)	0.19 (0.81)	0.27 (0.73)
7. <i>Centrarchus macropterus</i>	0.25 (0.25)	0.55 (0.30)	0.25 (0.25)	0.25 (0.25)	0.10 (0.10)	0.10 (0.10)		0.28 (0.72)	0.41 (0.59)	0.27 (0.73)
8. <i>Acantharchus pomotis</i>	0.25 (0.20)	0.40 (0.20)	0.25 (0.20)	0.25 (0.20)	0.10 (0.10)	0.10 (0.10)	0.40 (0.20)		0.27 (0.73)	0.37 (0.63)
9. <i>Ambloplites rupestris</i>	0.25 (0.30)	0.40 (0.25)	0.25 (0.30)	0.25 (0.30)	0.10 (0.10)	0.10 (0.10)	0.40 (0.25)	0.55 (0.20)		0.28 (0.72)
10. <i>Archoplites interruptus</i>	0.25 (0.15)	0.40 (0.15)	0.25 (0.15)	0.25 (0.15)	0.10 (0.10)	0.10 (0.10)	0.40 (0.15)	0.55 (0.15)	0.55 (0.15)	

¹ Above diagonal: allozyme-based genic similarities (distances) calculated from allele frequencies at 11–14 genetic loci, using Rogers' (1972) formulas. Below diagonal: similarities obtained from the "formal classification" of Centrarchidae as described in text; and in parenthesis, similarities obtained from the dendrogram in Fig. 3 based on Branson and Moore's (1962) study of the acoustico-lateralis system.

may be obtained (Fig. 3). Again, the range of similarities was chosen to roughly correspond to those based on the genic and "formal classification" estimates, and the range was divided equally by the number of branch points in the dendrogram. Estimates of similarity taken from this dendrogram have been entered in Table 3, and form another basis of comparison with the genic data. Of course we realize that the similarity estimates summarized for the "formal classification" and Branson and Moore data are imperfect, and may be criticized to detail. However, we are interested in main effects, and feel that attempts to objectively quantify information from various sources are necessary for comparative purposes.

In this section, we will describe the results of the genic information in relation to previously published accounts of the probable relationships of centrarchid genera.

Systematic relationships of Elassoma.—Jordan (1877) first described *Elassoma* and believed it to be a member of the Cichlidae. Berg (1946) considered *Elassoma* to belong to Centrarchidae, where it is presently placed. Most workers feel that ellassomids either diverged very early from

primitive centrarchid stock or else have completely different affinities. Branson and Moore (1962) argue that *Elassoma* should be raised to family status.

In the biochemical dendrogram (Fig. 2), *Elassoma* appears to be the most distinct of the centrarchid genera, joining the other genera at $S = 0.22$. It is conceivable that this low level of genic similarity is the result of chance identities in mobility of a few proteins, or even to convergence to a common allelic state at a few loci. Or perhaps the loci at which alleles appear to be

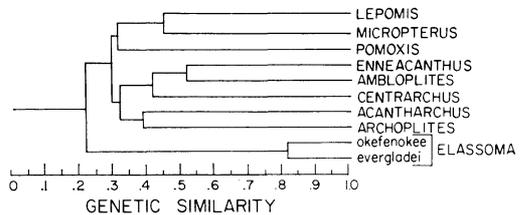


Fig. 2. Allozyme-based dendrogram of centrarchid genera based on unweighted pair-group cluster analysis of genic similarity coefficients derived from 11–14 genetic loci.

shared with some other species (**Ldh-1**, **Ldh-2**, **Idh-1**) are more slowly evolving. We cannot distinguish between these possibilities with the present data. It would be interesting to examine very distantly related fish (such as those belonging to different orders) to establish whether shared electrophoretic mobilities due to chance and convergence are common phenomena. At any rate, by all criteria *Elassoma* is the most distinct of centrarchid genera.

Three living species are currently assigned to *Elassoma*, and we have examined two of them. *Elassoma okefenokee* and *E. evergladei* are genically very similar to one another, $S = 0.82$. Nonetheless they are completely distinct in allelic composition at **Pgi-1** and **Pgi-2**. These two species were obtained from the same collecting site. Since two distinct gene pools are represented, the species status of these forms is confirmed, despite their overall biochemical affinity. These two species appear about as similar genically as do the two most closely related species of *Lepomis* (*L. marginatus* and *L. megalotis*, $S = 0.79$ —Avisé and Smith, 1974b).

Relationships within the Centrarchinae.—This subfamily contains two tribes, Ambloplitini and Centrarchini. In Ambloplitini are currently included *Archoplites*, *Ambloplites* and *Acantharchus*. *Acantharchus* and *Archoplites* cluster together in the biochemical dendrogram, notwithstanding the fact that they are genically quite different from one another, $S = 0.37$. However, *Ambloplites* appears genetically somewhat closer to *Enneacanthus* and not particularly close to the other Ambloplitini.

Archoplites, the Sacramento perch, is the only living sunfish native to waters west of the Rockies. Since sunfish are characteristic of lowland waters, *Archoplites* was probably separated from other centrarchids not later than the formation of the Rocky Mountains, which began during the Miocene or early Pliocene (Schuchert and Dunbar, 1941). Branson and Moore (1962) argue that the Sacramento perch was early isolated from other sunfish and "has become evolutionarily stagnated." Its placement in Ambloplitini may thus reflect its apparent retention, with *Ambloplites* and *Acantharchus*, of certain traits considered characteristic of primitive centrarchid stock, and may not necessarily reflect a recent shared ancestry. The biochemical evidence does not contradict this hypothesis.

Ambloplites is placed near *Acantharchus* and *Micropterus* by Schlaikjer (1937), near *Acantharchus*, *Centrarchus* and *Pomoxis* by Bailey (1938), and nearest *Acantharchus* by Branson

and Moore (1962). Nonetheless, Branson and Moore note that the relationship at best is an obscure one; again the retention of supposedly primitive characters rather than recent shared ancestry may explain its taxonomic affinity to *Acantharchus*. *Acantharchus* and *Ambloplites* show $S = 0.27$.

The tribe Centrarchini contains *Pomoxis* and *Centrarchus*. There is general agreement among most workers that these genera are more closely related to one another than to other centrarchid genera. This conclusion is not apparent from the genic data; *Pomoxis* and *Centrarchus* share alleles at only two of eleven loci, **Ldh-2** and **Mdh-1**, and overall biochemical similarity is only $S = 0.18$.

Relationships within the Lepominae.—Three monogeneric tribes are included in this subfamily, the Enneacanthini, Lepomini and Micropterini. The banded sunfish genus *Enneacanthus* is generally thought to be the most distinct member of this subfamily, with *Lepomis* (including the former *Chaenobryttus*) and *Micropterus* more closely related.

Lepomis is the most species-rich of centrarchid genera, and contains eleven living species. The warmouth, formerly *Chaenobryttus gulosus*, has recently been placed in the genus *Lepomis* (Bailey et al., 1970) on the basis of overall similarity and especially its ready ability to hybridize with other lepomine forms (Hubbs, 1955; West, 1970; West and Hester, 1966; Birdsong and Yerger, 1967). Hybrids between *L. gulosus* and either *L. cyanellus* or *L. macrochirus* are at least partially fertile (Childers, 1967; West, 1970). All ten *Lepomis* species examined cluster together in a biochemical dendrogram, distinct from all other genera (Avisé and Smith, 1974b).

Biochemically, *Micropterus* clusters closest to *Lepomis*, even though these genera are completely distinct in allelic composition at more than 50% of their loci ($S = 0.45$). *Micropterus* and *Lepomis* are thought to be fairly closely related on other grounds as well; in particular, *Lepomis gulosus* \times *Micropterus salmoides* hybrids are completely viable although sterile (West, 1970).

DISCUSSION

In order to objectively quantify the correspondence between postulated relationships of sunfish genera based on different criteria, we have calculated correlation coefficients between similarity estimates obtained from (A) genic information, (B) the acoustico-lateralis system, (C) the "formal classification," and (D) general

TABLE 4. CORRELATION COEFFICIENTS BETWEEN SIMILARITY ESTIMATES OF CENTRARCHID GENERA.¹

	A	B	C	D
A	—	0.45	0.22	0.23
B		—	0.46	0.05
C			—	0.05
D				—

¹ Similarity estimates based on (A) genic data from the present study, (B) the acoustico-lateralis system (Branson and Moore, 1962), (C) the "formal classification" of Centrarchidae, and (D) an early morphological study (Schlaikjer, 1937). Similarity values used to generate several of the correlations are given in Table 3.

morphology. These correlations are presented in Table 4.

The correlations between various sets of information are in all cases positive, but not strong. The highest correlation is that between Branson and Moore's (1962) study of the lateral line system and the "formal classification." This is not too surprising since, in fact, the formal classification in most cases follows the suggestions of Branson and Moore. What is surprising is that this correlation is not higher. There are at least two possible reasons for this. 1) As already mentioned, it appears that some genera are placed in the same tribe or subfamily primarily on the basis of sharing particular traits, for example primitive traits, rather than on the basis of a relatively recent shared ancestry. Yet the dendrogram (Fig. 3) used to estimate similarities between genera from Branson and Moore's work is based on the assumption that similarity is primarily a (negative) function of time since divergence from a common ancestor. Of course, the lack of close correspondence between phenetic similarity and time since divergence is a commonly reported phenomenon and forms the heart of the problem of determining a truly phylogenetic systematics. 2) It is likely that the similarity coefficients presented in Table 3 distort somewhat the results of various studies, due to the necessarily artificial and simplistic manner in which they were calculated. However, this potential bias should not favor any particular correlations over others, but likely lowers them all. If this is true, the relative levels of the correlation coefficients are of more significance than their absolute levels.

The allozyme based information correlates most strongly with information from the acoustico-lateralis system. Similarities are most apparent in 1) the clustering together of all *Lepomis* species, 2) the clustering of *Microp-*

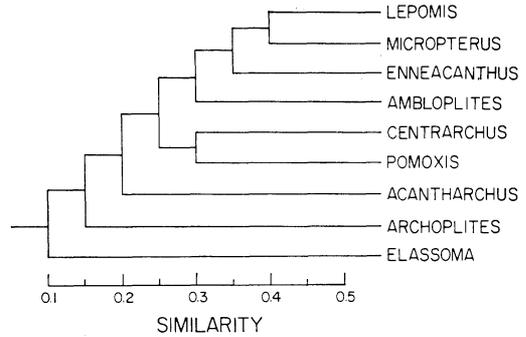


Fig. 3. Dendrogram of centrarchid genera based on a detailed study of the acoustico-lateralis system (from Fig. 11 of Branson and Moore, 1962).

terus with *Lepomis*, 3) the placement of *Acantharchus* with *Archoplites* and 4) the distant relationship of *Elassoma* to the other sunfish genera. The correlation between the formal classification and the allozyme data is somewhat lower, $r_{AC} = 0.22$. Nonetheless, this correlation is still considerably stronger than that calculated between another set of morphological data (taken from Schlaikjer, 1937) and either the acoustico-lateralis system ($r_{BD} = 0.05$) or the formal classification ($r_{CD} = 0.05$).

The mean level of genic similarity between the nine centrarchid genera is low, $\bar{S} = 0.29$. In other words, on the average, species belonging to different genera appear similar in allelic composition at only about 30% of their loci. And this is perhaps a conservative guess, since electrophoretic methods consistently show a bias towards underestimating the differences between species (Avisé, 1974). Clearly a very large number of genic differences have accumulated during the independent evolutionary histories of these fishes. Miller (1965) postulates that at least five living centrarchid genera had already made their appearance in the Miocene or at the Miocene-Pliocene boundary (*Pomoxis*, *Microp-terus*, *Lepomis*, *Chaenobryttus* and *Ambloplites*). Thus the considerable genetic divergence between a number of genera may have accumulated over a period of at least 20 million years.

How do genetic distances between centrarchid genera compare to distances representing more recent stages of evolutionary divergence in the Centrarchidae? Four levels of comparison are now available: 1) between at least twelve geographic populations within each of two bluegill subspecies, *Lepomis macrochirus macrochirus* and *L. m. purpurescens* (Avisé and Smith, 1974a); 2) between these two bluegill subspecies

(Avisé and Smith, 1974a); 3) between ten species within the genus *Lepomis* (Avisé and Smith, 1974b); and 4) between nine genera (present study). Mean levels of genetic similarity and distance (Rogers' coefficients) at each of these levels are as follows:

	\bar{S}	\bar{D}
1) geographic populations	0.97	0.03
2) subspecies	0.85	0.15
3) species	0.53	0.47
4) genera	0.29	0.71

These values cover a very large range, and in broad perspective are closely correlated with levels of evolutionary divergence.

ACKNOWLEDGMENTS

This work was supported by AEC grant AT (38-1)-310.

LITERATURE CITED

- ALLENORF, F. W. 1975. Genetic variability in a species possessing extensive gene duplication: genetic interpretation of duplicate loci and examination of genetic variation in populations of rainbow trout. Unpubl. Ph.D. thesis, Univ. of Wash.
- AVISE, J. C. 1974. Systematic value of electrophoretic data. *Syst. Zool.* 23:465-481.
- , AND M. H. SMITH. 1974a. Biochemical genetics of sunfish. I. Geographic variation and subspecific intergradation in the bluegill, *Lepomis macrochirus*. *Evolution* 23:42-56.
- . 1974b. Biochemical genetics of sunfish. II. Genic similarity between hybridizing species. *Amer. Nat.* 108:458-472.
- BAILEY, R. M. 1938. A systematic revision of the centrarchid fishes, with a discussion of their distribution, variations, and probable interrelationships. Unpubl. Ph.D. dissertation, Univ. of Michigan.
- , J. E. FITCH, E. S. HERALD, E. A. LACHNER, C. C. LINDSEY, C. R. ROBINS AND W. B. SCOTT. 1970. A list of common and scientific names of fishes from the United States and Canada. *Amer. Fish. Soc. Serial Pub.* 6.
- BERG, L. S. 1947. Classification of fishes both recent and fossil. Edwards Brothers, Inc., Ann Arbor, Michigan.
- BIRDSONG, R. S., AND R. W. YERGER. 1967. A natural population of hybrid sunfishes: *Lepomis macrochirus* × *Chaenobryttus gulosus*. *Copeia* 1967:62-71.
- BRANSON, B. A., AND G. A. MOORE. 1962. The lateralis components of the acoustico-lateralis system in the sunfish family Centrarchidae. *Copeia* 1962:1-108.
- CHILDERS, W. F. 1967. Hybridization of four species of sunfishes (Centrarchidae). *Bull. Illinois Natur. Hist. Surv.* 29:159-214.
- HESTER, F. E. 1970. Phylogenetic relationships of sunfishes as demonstrated by hybridization. *Trans. Amer. Fish. Soc.* 1970:100-104.
- HUBBS, C. L. 1955. Hybridization between fish species in nature. *Syst. Zool.* 4:1-20.
- JORDAN, D. S. 1877. Contributions to North American ichthyology based primarily upon the collections of the U.S. National Museum. *Bull. U.S. Natl. Mus.* 1877:50-51.
- MAY, B. 1975. Electrophoretic variation in the genus *Oncorhynchus*: the methodology, genetic basis, and practical applications to fisheries research and management. Unpubl. Masters Thesis, Univ. of Wash.
- MILLER, R. R. 1965. Quaternary freshwater fishes of North America, p. 569-581. In: *The Quaternary of the United States*. H. E. Wright and D. G. Frey (eds.) Princeton Univ. Press, Princeton, New Jersey.
- ROBERTS, F. L. 1964. A chromosome study of twenty species of Centrarchidae. *J. Morph.* 115:401-418.
- ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. *Studies in Genetics VII*. Univ. Texas Pub. 7213:145-153.
- ROMER, A. S. 1966. Vertebrate paleontology. Univ. Chicago Press, Chicago.
- SCHLAIKJER, E. M. 1937. New fishes from the continental Tertiary of Alaska. *Bull. Amer. Mus. Natur. Hist.* 74:1-23.
- SCHUCHERT, C., AND C. O. DUNBAR. 1941. A textbook of geology. Part II—Historical geology. John Wiley and Sons, New York.
- SELANDER, R. K., AND D. W. KAUFMAN. 1973. Genic variability and strategies of adaptation in animals. *Proc. Nat. Acad. Sci.* 70:1875-1877.
- , M. H. SMITH, S. Y. YANG, W. E. JOHNSON AND J. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). *Studies in Genetics VI*. Univ. Texas Pub. 7103:49-90.
- SICILIANO, M. J. 1973. Evidence for multiple unlinked genetic loci for isocitrate dehydrogenase in fish of the genus *Xiphophorus*. *Copeia* 1973:158-161.
- SOKAL, R. R., AND P. H. A. SNEATH. 1963. Principles of numerical taxonomy. W. H. Freeman, San Francisco.
- WEST, J. L. 1970. The gonads and reproduction of three intergeneric sunfish (family Centrarchidae) hybrids. *Evolution* 24:378-394.
- , AND F. E. HESTER. 1966. Intergeneric hybridization of centrarchids. *Trans. Amer. Fish. Soc.* 95:280-288.
- WHITT, G. S., W. F. CHILDERS AND P. L. CHO. 1973. Allelic expression at enzyme loci in an intertribal hybrid sunfish. *Journ. of Heredity* 64:54-61.

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF GEORGIA, ATHENS, GEORGIA 30602, DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA 94720, SAVANNAH RIVER ECOLOGY LABORATORY, DRAWER E, AIKEN, SOUTH CAROLINA 29801. Accepted 5 May 1976.