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BIOCHEMICAL SYSTEMATICS IN SOUTHEASTERN POPULATIONS OF *Fundulus heteroclitus* AND *Fundulus grandis*

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ABSTRACT: Four populations of *Fundulus heteroclitus* from the southeastern United States were compared electrophoretically to eight populations of *F. grandis* from Florida by using the products of 25 loci. Significant differences were found between *F. heteroclitus* and *F. grandis*. Only minor variation was found among the eight populations of *F. grandis*, and the recognition of Gulf, Florida east-coast and Florida Keys populations as distinct species or subspecies was not supported. Therefore, the Florida Keys populations of *F. g. saguanus* are relegated to *F. g. grandis*. The name *F. g. saguanus* should apply, for now, only to Cuban material.

Atheriniform fishes of the *Fundulus heteroclitus*-*Fundulus grandis* species complex occur in tidal lagoons and estuaries along the North American coastline from Labrador to Yucatan. *Fundulus heteroclitus* (Linnaeus), the mummichog, ranges from Labrador to northeastern Florida (Relyea, 1983). This is an especially well studied species, and the reader is referred to a recent American Society of Zoologists symposium (1986) devoted to its biology. Relyea (1983) described the current taxonomy of this species. *Fundulus heteroclitus* is sympatric with *F. grandis* in northeast Florida. The two species can be distinguished by the eight mandibular pores and long ovipositor of *F. heteroclitus*

versus ten mandibular pores and short ovipositor of *F. grandis*. There are also differences in body shape, coloration, and other meristic features.

The Gulf killifish, *Fundulus grandis* Baird and Girard, ranges from northeastern Florida southward to the Florida Keys and along the Gulf of Mexico coast from Florida to at least Veracruz, Mexico and disjunctly in Cuba (Rivas, 1948; Relyea, 1983). Within that range two subspecies are recognized, *Fundulus grandis saguanus* in Cuba, extreme southern Florida and the Florida Keys (Rivas, 1948, Relyea, 1983), and *F. grandis grandis* elsewhere (Miller, 1955). In extreme southeast and southwest Florida, there is an apparent absence of *F. grandis*

(Relyea, 1983). *Fundulus g. saguanus* therefore seems to be allopatric with respect to other *F. grandis* populations, and bisects the range of *F. g. grandis* into two disjunct populations, one in the Gulf of Mexico and one on the east coast of Florida. Relyea (1983) noted that *F. g. saguanus* possibly could be a distinct allopatric species, but commented that the distinguishing traits of *F. g. saguanus* could be ecophenotypically plastic and expressed under the unique environmental conditions of the Florida Keys and Cuba. These traits, therefore, may not represent genetic divergence but instead represent the effect of the environment on the expression of the genotype. A similar situation was seen in the atheriniform fishes of the genus *Menidia* (Duggins *et al.*, 1986). In addition, Gulf and Florida east-coast populations could be similar morphologically through convergence and yet be genetically distinct. Relyea (1983), who questioned the recognition of subspecies of killifishes in Florida, nevertheless recognized *F. g. saguanus* because of its traditional status and called for more study.

In this paper, we analyze by means of allozyme electrophoresis Florida populations of *F. grandis* and populations of *F. heteroclitus* from southeastern North Carolina and northeastern Florida. Biochemical characters provide an alternative data base for assessing divergence and relationships for these fishes.

MATERIAL EXAMINED

Between June, 1985 and July, 1986, 295 individuals from 12 localities (Figure 1) were collected. Collections were made with a 5-m seine, dipnet, or castnet. The fish were frozen on a block of dry ice until returned to the laboratory. Once in the laboratory, the specimens were stored at

-90°C until used for electrophoresis. When collected, all fishes from sympatric NE Florida localities were frozen together and then separated in the laboratory according to species using the characters ovipositor length, number of mandibular pores, body shape and coloration.

LOCALITIES

Fundulus heteroclitus: North Carolina: population number 1) Brunswick County, Wrightsville Beach, 20 specimens. Florida: 2) St. Johns Co., Matanzas Inlet, 20; 3) St. Johns Co., Marineland vicinity, 20; 4) Flagler Co., Rte. 100 at intercoastal waterway, 24. *Fundulus grandis*: Florida: 5) St. Johns Co., Marineland vicinity (same site as population 3), 20; 6) Brevard Co., Patrick AFB vicinity, 36; 7) Monroe Co., Key Largo, 23; 8) Monroe Co., Big Pine Key, 20; 9) Collier Co., Marco Is., 36; 10) Pinellas Co., Desoto

Figure 1. Florida populations of *Fundulus heteroclitus* (2-4) and *F. grandis* (5-12) examined in this study. Population 1, Wrightsville Beach, North Carolina, not shown; map of Florida omits panhandle west of the Appalachian R.

Co. Park, 20; 11) Dixie Co., Shired Island, 36; 12) Franklin Co., Carrabelle, 20.

METHODS

To obtain protein samples for electrophoresis, either entire fish were homogenized (SL < 30 mm) in an equal volume of chilled distilled water, or in larger fish, an eyeball, the heart, liver and a sample of skeletal muscle were homogenized (together) in an equal volume of chilled distilled water. In either case, electrophoretic patterns were the same. The slurry that resulted was centrifuged at 25,000g at 4° C for 60 min. The supernatant was decanted and stored at 4° C overnight, a maximum of 18 hours prior to electrophoretic separations. At the onset of this study, the identities of individual loci were determined by electrophoresis of individual tissue extracts from eye, muscle and liver.

The 25 loci coding for proteins surveyed in this study were: nonenzymatic proteins (Gp-1, 2, 3, 4); aspartate aminotransferases (S-Aat-A,B); esterase (Est-1); glucose-6-phosphate isomerases (Gpi-A,B); glyceraldehyde-3-phosphate dehydrogenases (Gadph-A,C); alpha glycerophosphate dehydrogenase (∂ Gpdh-A); isocitrate dehydrogenase (M-Idh-A); 1-iditol dehydrogenase (Iddh-A); lactate dehydrogenase (Ldh-A); malate dehydrogenases (NAD dependent) (M-Mdh-A, S-Mdh-A); malate dehydrogenases (NADP dependent) (Me-A,B); mannose-6-phosphate isomerase (Mpi-A); peptidases (Pep-1,2); phosphogluconate dehydrogenase (Pgd-A); and phosphoglucotases (Pgm-A,B).

Techniques of starch gel electrophoresis were as in Duggins *et al.* (1983). Gp, Est, Gpi and Mpi were resolved on the LiOH buffer described by Selander *et al.* (1971). The remaining loci were resolved on a tris citrate (pH 8) buffer

(Selander *et al.*, 1971). All gels were 12% starch (Electrostarch, Lot 392, Otto Hiller Electrostarch Co., Madison, Wisconsin).

The locus nomenclature system follows Fisher *et al.* (1980) and Crabtree and Buth (1981). When allelic variation occurred, the allele with the greatest anodal migration was called *a*, the next *b*, and so on. Electrophoretic data were analyzed with BIOSYS-1 (Swofford and Selander, 1981) on the UALR-DEC-VAX 11/780 computer cluster.

RESULTS

Of the 25 loci examined, 12 were essentially monoallelic (most common allele occurring at a frequency of 0.95 or greater). These loci were Me-B, S-Aat-B, M-Mdh-A, Gadph-A, C, Ldh-A, Pgd-A, Iddh-A, and Gp-1-4. At Mpi, Pgm-B, Pep-1, 2 and M-Idh-A only minor variation from a most common allele (>0.78) was found. Greater variation occurred at the remaining eight loci. Genotype arrays are provided for these loci in Table 1. The genotype arrays were tested for conformance with Hardy-Weinberg equilibrium expectations. Statistically significant deviation from expectations were found at Gpi-A, populations 3 and 11 (*F. heteroclitus*, Marineland, *F. grandis*, Shired Island), Gpi-B, population 10 (*F. grandis*, Desoto), ∂ Gpdh-A, population 5 (*F. grandis*, Marineland), Me-A, populations 1, 3 and 4 (*F. heteroclitus*, Wilmington and Marineland, *F. grandis*, Rte 100), and Pgm-A, populations 1 and 8 (*F. heteroclitus*, Wilmington, and *F. grandis*, Big Pine Key).

DISCUSSION

When standard genetic distance values (modified Rogers distance, Wright, 1978) were used to construct a phenogram (Figure 2; UPGMA, Sneath

Table 1. Genotypic distributions for 8 loci for 4 populations of *F. heteroclitus* and 8 populations of *F. grandis*. The number of individuals of each genotype is provided in parentheses.

Locus	Populations											
	<i>Fundulus heteroclitus</i>				<i>Fundulus grandis</i>							
	1 Willm	2 Matanzas	3 Mrlld	4 Rte 100	5 Mrlld	6 Cocoa	7 K. Largo	8 BPK	9 Marco	10 Desoto	11 Shired	12 Crbl
Est-1	cc (20)	cc (19) cd (1)	cc (14) cd (6)	bc (1) cc (19)	cc (2) cd (10) dd (8)	cc (27) cd (8) dd (1)	cc (23)	cc (20)	cc (36)	cc (20)	bc(2) cc (34)	cc (20)
Gpi-A	cc (12) cd (8)	cc (12) cd (6) dd (2)	cc (13) cd (4) dd (3)	bc (1) cc (13) cd (7) dd (3)	bc (1) cc (19)	cc (36)	ac (1) cc (19) cd (3)	cc (20)	cc (29) cd (7)	ac (1) cc (15) cd (3) ce (1)	aa (1) ac (1) cc (33) cd (1)	cc (19) ce (1)
Gpi-B	bb (20)	bb (18) bc (2)	ab (1) bb (16) bc (3)	ab (2) bb (19) bc (2) cc (1)	bc (5) cc (15)	cc (36)	bc (1) cc (19)	cc (20)	cc (36)	bb (1) bc (1) cc (18)	bc (1) cc (35)	cc (20)
βGpdh	bc (1) cc (18) cd (1)	cc (20)	cc (20)	bc (1) cc (23)	bb (2) bc (3) cc (15)	bb (4) bc (21) cc (11)	bc (3) cc (18) cd (2)	cc (16) cd (4)	cc (36)	cc (20)	cc (36)	cc (20)
Me-A	cc (17) cd (1) dd (2)	cc (20)	cc (13) cd (3) dd (4)	cc (9) cd (5) dd (10)	dd (20)	dd (36)	dd (20)	dd (20)	dd (36)	dd (20)	dd (36)	dd (20)
Pgm-A	bb (2) bc (1) cc (10) cd (4) dd (2) de (1)	bc (1) bd (2) be (1) cc (7) cd (3) ce (1) dd (5)	bc (1) bd (5) be (1) cc (3) cd (6) ce (1) dd (3)	bc (2) cc (8) cd (8) ce (3) dd (3)	cc (18) cd (2)	cc (33) cd (3)	cc (17) cd (3) ce (3)	bb (1) bc (1) cc (6) cd (5) ce (1) dd (2) de (4)	ac (1) ad (1) bc (6) bd (4) cc (13) cd (8) ce (1) dd (2)	bc (1) bd (1) cc (5) cd (10) dd (3)	bc (1) bd (1) cc (18) cd (8) ce (1) dd (3) de (3) ee (1)	bc (4) cc (10) cd (5) dd (1)
S-Aat-A	bb (3) bc (8) cc (9)	bc (1) cc (19)	cc (20)	cc (24)	cc (24)	cc (36)	cc (20)	cc (20)	cc (36)	cc (20)	cc (36)	bc (1) cc (19)
S-Mdh-A	cc (20)	bc (1) cc (19)	bc (1) cc (19)	bc (1) cc (23)	cc (20)	bc (1) cc (34) cd (1)	cc (20)	cc (20)	bc (8) cc (28)	cc (20)	ac (1) bc (14) cc (21)	bc (1) cc (19)

and Sokal, 1973), two distinct clusters resulted: a *Fundulus heteroclitus* cluster (populations 1-4) and a *F. grandis* cluster (populations 5-12).

The genetic data presented here support previous morphological and meristic data (Relyea, 1983) on which the recognition of *F. heteroclitus* and *F. grandis* is based. These two species show a nearly fixed difference at Gpi-B

and a marked difference at Me-A. At a locality where both species were collected in approximately equal percentages in the same seine haul (Marineland, populations 3 and 5) these differences are maintained. Other loci at this locality (Est-1, Gpi-A, and Pgm-A) show statistically significant frequency differences between the two species (Chi square test, $p = 0.05$, Goodnight *et al.*,

Figure 2. Phenogram generated with genetic distance statistics using modified Rogers distance (Wright, 1978).

1982). We caution, however, that our sample size is small (20 specimens of each species).

We cannot rule out hybridization between these two species in north-eastern Florida; in fact, our data indicate that possibility as the two species have alleles in common in the area of sympatry. A definitive hybrid would be difficult to demonstrate since there are no absolutely fixed differences at any of the loci examined.

Analysis of the *F. grandis* cluster suggests that Florida Keys populations (nominally *F. g. saguanus*) cluster with Gulf populations (Figure 2). The percentage of polymorphic loci (most common allele < 0.95) was lower in Florida Keys populations (Table 1). The genetic distances indicated in Figure 2 show all populations of *F. grandis* to be very closely related, and surely belong to a single species.

Given the small genetic distances and the pattern of geographic variation between populations, we do not support the recognition of Gulf, Florida east coast and Florida Keys populations of *Fundulus grandis* as distinct species nor

subspecies. Although geographic variation is apparent, it is not as marked as for morphological traits (Relyea, 1983) which are likely to be environmentally labile in their expression. This situation in *Fundulus* parallels the case in *Menidia* (Chernoff et al., 1981; Duggins et al., 1986).

The status of *F. g. saguanus* is still unresolved because the type-series includes only Cuban specimens (Rivas, 1948); we recommend tentatively that the name apply only to Cuban material. Although perhaps justifiably recognizable as *F. g. saguanus* on a morphological basis, Florida Keys populations cannot be separated from *F. g. grandis* by biochemical techniques. The same situation occurs in Florida Keys populations of *Menidia peninsulae*, formerly *M. conchorum*, which differ in meristic features from the peninsular Florida *M. peninsulae*, but have not been shown to differ by biochemical techniques (Duggins et al., 1986). These observations underscore the need for serious experimental investigation of environmental influences on morphometric and meristic variation in atheriniform fishes.

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