Biochemical Systematics and Ecology, Vol. 14, No. 2, pp. 233–238, 1986.
Printed in Great Britain.

0305–1978/86 $3.00+0.00
Pergamon Press Ltd.

An Intergeneric Hybrid, *Campostoma anomalum* × *Rhinichthys atratulus*, from the Youghiogheny River Drainage, West Virginia

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Key Word Index—*Campostoma anomalum*; *Rhinichthys atratulus*; Cyprinidae; Cypriniformes; isozymes; acrylamide electrophoresis; hybrid; morphometric-meristic.

Abstract—The first record of a natural intergeneric hybrid of *Campostoma anomalum* × *Rhinichthys atratulus* is described from the Youghiogheny River drainage. Morphometric and meristic characters of the parents and hybrid were used to calculate a mean hybrid index value of 49.8. Dentition of the hybrid was intermediate as was intestinal coiling. In addition to the hybrid index, *C. anomalum*, *R. atratulus* and the hybrid were examined electrophoretically. Of the 32 enzymatic loci resolved, six (ADH-A, EST-B, LDH-A, PGM-A, SDH-B and SOD-C) showed hybrid inheritance patterns identical to the enzyme pattern obtained by mixing tissue extracts composed of the two parental species.

Introduction

Numerous cases of natural intergeneric cyprinid hybrids have been reported in the literature. Schwartz [1, 2] lists nine intergeneric hybrids of *Campostoma anomalum*. However, *Rhinichthys atratulus* has never been reported to hybridize naturally. Laboratory produced offspring of *R. atratulus* × *R. cataractae* [3–5] as well as *R. atratulus* × *Semotilus atromaculatas* [6] have been reported. Howell and Villa [5] found no apparent gross karyotypic differences which might account for the absence of natural *R. atratulus* × *R. cataractae* hybrids. Bartnik [4] suggested that the barriers to natural hybridization of *R. atratulus* are behavioral and ecological, not genetic.

Morphological character analysis is typically used to identify the parental species of fish hybrids, assuming that the hybrids are morphologically intermediate between the parental species [6]. Hubbs [7] claimed that natural interspecific hybrids typically demonstrate intermediacy between their parental species, while Smith [8] and Neff and Smith [9] stress caution in assuming that phenotypic expressions in hybrids are consistently intermediate. Recently, hybrids have been identified using electrophoretic techniques [10]. Because of the possible problems associated with the misidentification of parental species using morphological and meristic techniques, electrophoretic techniques were used as an additional diagnostic tool. This paper describes morphological and biochemical analyses, of the hybrid *C. anomalum* × *R. atratulus* from the Youghiogheny River drainage.

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(*Received* 5 *December* 1984)

Results

Table 1 summarizes data for 12 morphometric and five meristic characters. Hybrid indices are intermediate (30–70) for five characters, closer to *Campostoma anomalum* in three characters and closer to *Rhinichthys atratulus* in three characters. All hybrid characters were within the range for both parents; however, some mean characters may be considered as 'extremes' in that they are outside the range of mean values determined for the parental species. Eye diameter and least

TABLE 1. COMPARISON OF MORPHOMETRIC AND MERISTIC CHARACTERISTICS OF THE HYBRID *CAMPOSTOMA ANOMALUM*×*RHINICHTHYS ATRATULUS* WITH ITS PUTATIVE PARENTS

Character	*C. anomalum* *N*=25 Range	$\bar{X}$	Hybrid *N*=4 Range	$\bar{X}$	*R. atratulus* *N*=25 Range	$\bar{X}$	Hybrid index
Standard length (mm)	51.9–109.1	82.1	46.6–58.2	51.3	43.2–67.4	58.6	—
Thousands of standard length							
Head length	224–288	241	231–266	251	233–272	252	90.9
Head depth	126–174	151	134–154	143	121–148	133	44.4
Body depth	192–253	231	177–203	193	187–239	215	*
Snout to dorsal	481–543	506	497–529	517	529–582	560	20.4
Snout to pelvic	430–480	461	470–497	482	470–514	492	67.7
Snout length	82–106	91	75–92	85	74–92	82	66.7
Eye diameter	38–54	45	49–66	57	45–69	54	†
Least caudal peduncle depth	97–120	109	109–122	117	100–128	111	†
Thousands of head length							
Snout length	310–450	383	325–345	337	301–359	326	80.1
Eye diameter	146–230	188	196–257	226	182–278	215	†
Thousands of snout length							
Eye diameter	401–606	493	569–767	672	550–877	662	†
Lateral line scales	47–57	49.0	56–58	57.3	59–84	68.1	43.4
Scales above L.L.	6–9	7.0	8–9	8.7	10–14	12.6	30.4
Scales below L.L.	6–8	6.2	6	6.0	6–8	7.0	*
Scales around caudal peduncle	10–12	11.7	15–17	16.3	16–20	17.5	79.3
Number of barbels	0	0	0–2	0.5	2	2.0	25.0
Cartilaginous plate (0=absent; 1=present)	1.0	1.0	1.0	1.0	0	0.0	0.0

*Hybrid value less than the mean for either parent.
†Hybrid value greater than the mean for either parent.

caudal peduncle depth were greater than the mean for either parent while body depth and scales below the lateral line were less than the mean for either parent. The mean hybrid index, exclusive of these extreme characters, was 49.8. Dentition was also intermediate—the four hybrid specimens were 1, 4-4, 1; while *C. anomalum* was 0, 4-4, 0 (*N*=23) and 1, 4-4, 0 (*N*=2) and *R. atratulus* was 2, 4-4, 2 (*N*=25).

Intermediate intestine coiling occurs in this hybrid as compared to the herbivorous *C. anomalum* (long coiled intestine) and the omnivorous *R. atratulus* (short S-shaped intestine). Other more qualitative morphometric characteristics of the hybrid (Fig. 1) appear to be intermediate to the putative parents. The position of the lateral line is intermediate to the straight lateral line of *C. anomalum* and the curved lateral line of *R. atratulus*. The shape of the head and position of the mouth, as well as the size of scales, are intermediate when compared to the parents. The hybrid also possesses a lower jaw plate characteristic to *C. anomalum*. Pigmentation of the hybrid appears to be closer to *R. atratulus*, with the most prominent characteristic being the dark lateral band.

To assist in the identification of the parents of the hybrid and hybrid verification, electrophoretic techniques were employed. Each specimen (23 individuals) was analysed for gene products of 32 loci, resolving 38 enzymatic alleles. Six loci, alchohol dehydrogenase A (ADH-A), esterase-B (EST-B), lactate dehydrogenase-A (LDH-A), phosphoglucomutase-A (PGM-A), sorbitol dehydrogenase-B (SDH-B) and superoxide dismutase-C (SOD-C) showed hybrid inheritance patterns (Fig. 2). These inheritance patterns were always additive (intermediate) in that if the two parental species had distinct alleles occurring at a locus, then the hybrid expressed both alleles (Fig. 2). In all cases, the pattern of the hybrid was identified to the enzyme pattern obtained by mixing tissue extracts composed of the two parental species.

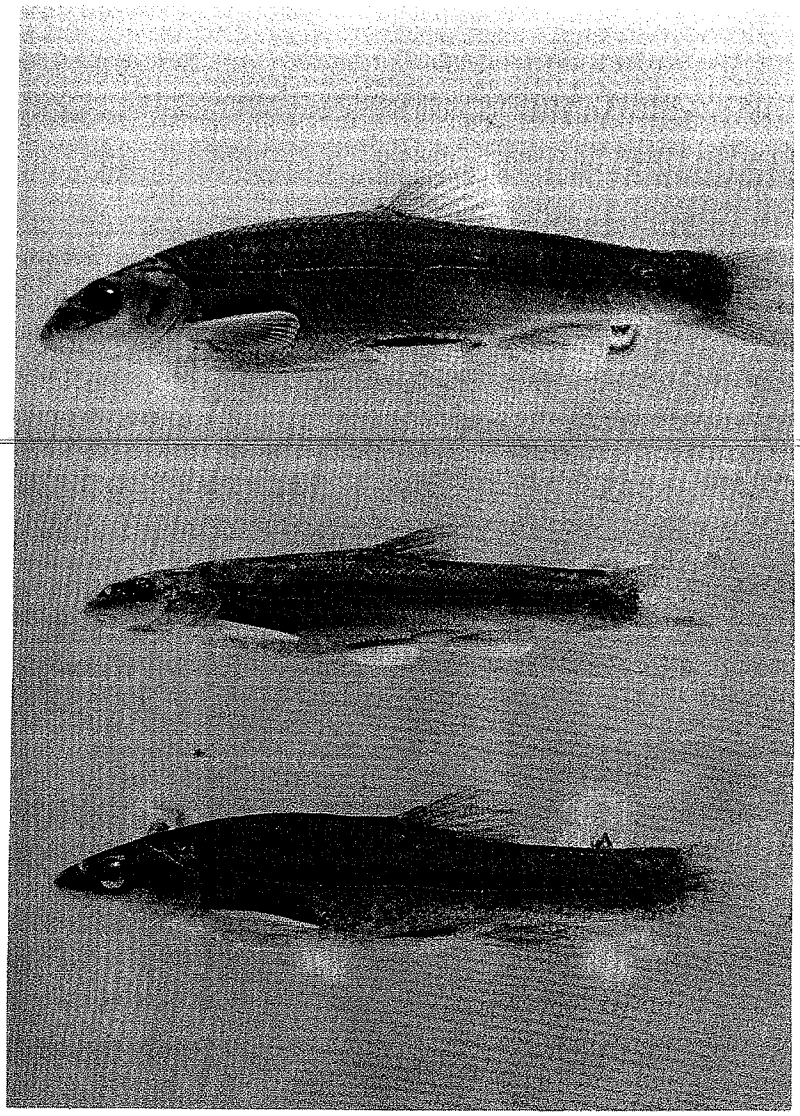

FIG. 1. LATERAL VIEW (TOP TO BOTTOM) OF *CAMPOSTOMA ANOMALUM*, HYBRID AND *RHINICHTHYS ATRATULUS*.

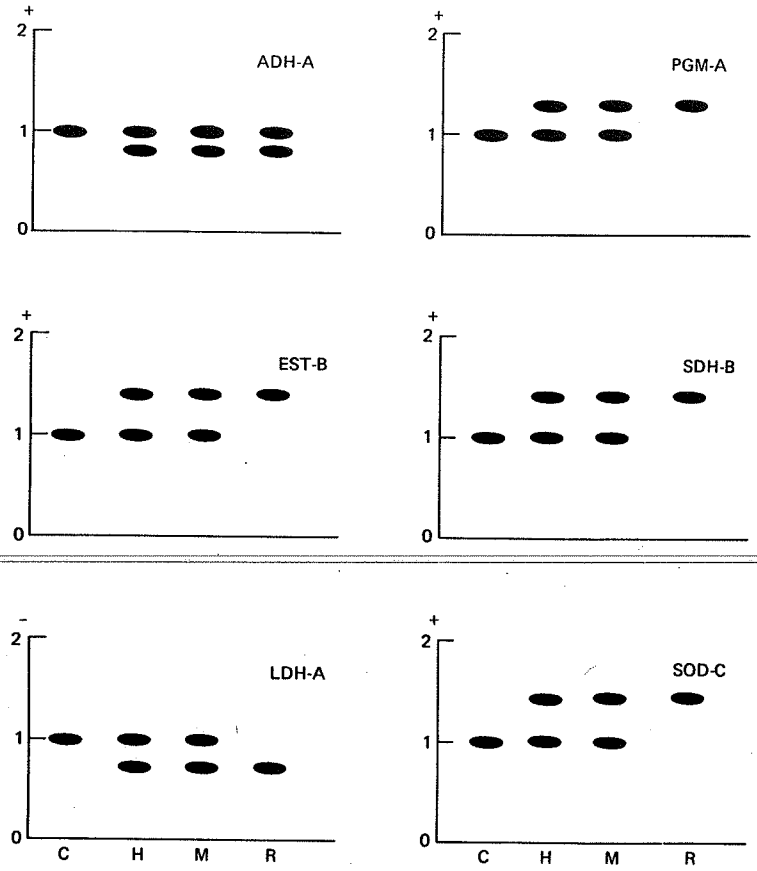

FIG. 2. ELECTROPHORETIC MOBILITIES OF ENZYMES AMONG *CAMPOSTOMA ANOMALUM* (C), THE HYBRID (H), THE MIXTURE (M) OF *C. ANOMALUM* AND *RHINICHTHYS ATRATULUS* TISSUE EXTRACTS AND *R. ATRATULUS* (R).

Discussion

Campostoma anomalum and *R. aratulus* are widespread throughout much of North America [11]. The occurrence of this intergeneric hybrid was probably the result of accidental fertilization. Both *C. anomalum* and *R. atratulus* have been observed spawning over *Nocomis* nests [12]. Both species have been reported to spawn from late April to June depending on the locality [13]. Peak spawning temperatures also overlap with *C. anomalum* reported as 16–21° and *R. atratulus* as 18–22° [13].

This hybrid represents the first natural intergeneric hybrid with *R. atratulus* as a putative parent. Using a traditional hybrid index, the hybrid had a mean index value of 49.8. In addition, electrophoretic techniques showed six loci with intermediate electromorphs. Bartnik [4] suggested that the barriers to natural hybridization involving *R. atratulus* are most likely behavioral and ecological, not genetic. The cohabitation of a rare and abundant fish have been suggested as catalysts for hybridization [14, 15], as has the crowding of spawning fish due to limited spawning habitat [14, 16]. In this particular situation, populations of *R. atratulus* in Snowy Creek are small with limited spawning habitat. In the 100 m of stream sampled, approximately 25 *Nocomis* were observed. Both *R. atratulus* and *C. anomalum* most likely spawned over existing *Nocomis* nests, due to the limited spawning habitat.

Experimental

Three specimens of the hybrid *Campostoma anomalum*× *Rhinichthys atratulus* were taken from the Youghiogheny River drainage, Snowy Creek, at the spillpool of Terra Alta Lake and 100 m downstream; Preston County, West Virginia. Individuals used in electrophoretic analysis were transported live, back to the Appalachian Environmental Laboratory and stored at −80° before analysis. Fishes used for morphometric and meristic measurements were previously collected from the same locality and stored in 40% isopropanol at the Appalachian Environmental Laboratory Fish Museum. One additional hybrid, collected from the same locality but misidentified as *R. atratulus*, was discovered in the museum. Of these specimens two were males and two were females.

Twenty-five *C. anomalum* and *R. atratulus* were compared to the four hybrids. Morphometric characters were measured to the nearest 0.1 mm with dial calipers [17]. A hybrid index was calculated [18]:

$$H=[(X_H-\mu_1)/(\mu_2-\mu_1)]\times 100$$

where H=hybrid index, X_H=hybrid value, μ_1=value for *C. anomalum* and μ_2=value for *R. atratulus*. An index value of 50 denotes exact intermediacy; less than 50 indicates that the particular character is closer to *C. anomalum*, and over 50 indicates a closer affinity with *R. atratulus*.

A total of 10 *C. anomalum*, 10 *R. atratulus* and three hybrids were examined electrophoretically following Goodfellow *et al.* [10]. To determine if the hybrid had the same allelic composition of the putative parents, mixtures were prepared using equal parts of tissue extracts obtained from *C. anomalum* and *R. atratulus*. Histochemical staining procedures followed those described by Allendorf *et al.* [19].

Acknowledgements—This research was partially supported by the United States Forest Service through Grant 53-56A1-0-00372. Field assistance from the following individuals is gratefully acknowledged: E. Esmond, M. Hendricks and P. McKeown, who also assisted in laboratory preparation. This is Scientific Series No. 1631-AEL from the University of Maryland Center for Environmental and Estuarine Studies.

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