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HYBRIDIZATION BETWEEN *ETHEOSTOMA ZONALE* AND *ETHEOSTOMA OLMSTEDI* (TELEOSTEI: PERCIDAE), FOLLOWING AN INTRODUCTION EVENT.—Hybridization is known to occur more frequently in fishes than in most other vertebrate groups (Hubbs, 1955). Among North American freshwater fishes, the catostomids, cyprinids, poeciliids, and centrarchids (particularly sunfishes) commonly hybridize in nature (Hubbs and Bailey, 1940; Hubbs, 1955; Gilbert, 1961). Others, such as the darters, form hybrids only rarely (Page, 1983) and then only under a restricted set of conditions.

Schwartz (1972) listed 171 different darter hybrids. The majority (90%) were artifically reared in aquaria. Of the 17 hybrid combinations found in nature, most were from environments modified in some way. Natural hybridization events recorded for darters involve species with primitive reproductive behaviors (Hubbs, 1955). With the exception of interbreeding between subspecies of *Etheostoma nigrum,* none of the hybrid combinations listed by Schwartz includes species with advanced reproductive behavior (Page, 1985). Recently, Page (1975) reported a hybrid between *E. kennicotti* and *E. squamiceps,* two species with advanced (egg-clustering) behaviors.

Hubbs et al. (1943), and subsequent studies by Hubbs and Hubbs (1947) and Hubbs and Miller (1953), outlined a number of conditions conducive to hybridization. One of these conditions, the co-occurrence of two species in an area where one species is rare and the other species is abundant, may be realized following introduction events. Several studies have shown evidence for hybridization following an introduction (Nelson, 1973; Hocutt and Hambrick, 1973).

This paper describes a series of putative hybrids collected in the Susquehanna River, Pennsylvania, between 1971 and 1987. The purported parental species are the tessellated darter, *E. olmstedi,* and the banded darter, *E. zonale. Etheostoma olmstedi* is native to the Susquehanna River, while *E. zonale* is introduced.

The banded darter was first collected from the Susquehanna River drainage in 1971 in Little Pine Creek (Knieb, 1972). Collections from this area in 1963–64 yielded no banded darters (Cooper, 1983), indicating that the introduction probably occurred in the late 1960s. In 1972, *E. zonale* was collected more than 400 km downstream from the site where it was first observed (Denoncourt et al., 1975). It is currently the most abundant darter in many localities throughout the Susquehanna drainage.

We recorded 28 mensural and 18 meristic characters from seven putative hybrids and from 30 individuals of each of the purported parental species. Counts of serially repeated elements followed Hubbs and Lagler (1958). Included with this standard set of meristic data were five additional variables. Breast squamation and cheek squamation were recorded using the scale published by Tsai and Raney (1974) for *E. zonale.* Pigmentation pattern was scored from 1–3 with 1 = barring, 2 = intermediate, and 3 = "W-shaped" lateral blotches. The shape of the branchiostegal membrane was recorded as broadly joined, intermediate, or narrow. Palatine teeth were recorded as absent, rudimentary, or present. Mensural information was obtained using a truss network as described by

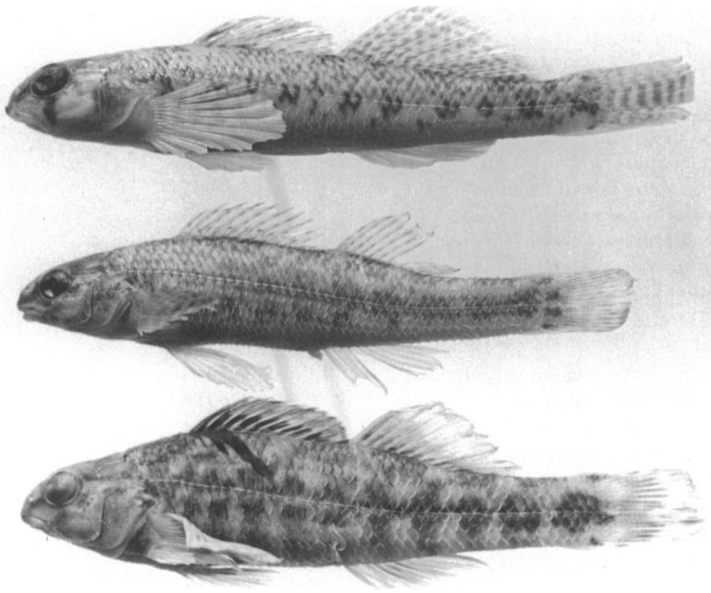

Fig. 1. Putative parental species *Etheostoma olmstedi* (top) and *E. zonale* (bottom) and their putative hybrid (center).

Humphries et al. (1981) and Bookstein et al. (1985). We also measured standard length, head length, and orbit diameter. Measurements were made with dial calipers to the nearest 0.1 mm.

Morphometric and meristic data were analyzed separately using principal components analysis (PCA). Sheared PCA (Humphries et al., 1981) was performed on the mensural data to allow for the comparison of shape independent of size. PCA does not require a priori identification of groups and is purely descriptive (Harris, 1975; Neff and Smith, 1979). Because intergroup differences are not weighted, problems associated with placing individuals into a priori groups on the basis of a few characters are eliminated (Neff and Smith, 1979), making PCA the appropriate analytical method for studies of hypothesized hybridization. Shearing, however, requires the designation of groups in order to calculate a within-group size component. The shearing procedure was performed with a SAS program written and supplied to us by D. L. Swofford.

Specimens identified as hybrids (Fig. 1) have an intermediate pigmentation pattern, no suborbital bar (a characteristic of both purported parents), a branchiostegal membrane that is intermediate between the broadly joined membrane of *E. zonale* and the more deeply incised membrane of *E. olmstedi*, and poorly formed palatine tooth patches. Palatine teeth are present and well developed in *E. olmstedi* but absent in *E. zonale*. The last two characters are not present in all specimens, however; two putative hybrids have broadly joined branchiostegal membranes, and two lack palatine teeth.

A plot of the principal component scores of all individuals on sheared principal component II (PCII, distance measures) vs principal component I (PCI), (meristic variables) shows a distinct cluster for *E. olmstedi* (Fig. 2). Scores of putative hybrids were much closer to those of *E. zonale*, with one individual at the periphery and one well within the cluster of *E. zonale*.

Variable loadings for sheared PC II and PC I are shown in Tables 1 and 2, respectively. Sheared PC II is primarily associated with the length of unpaired fins. A high score on sheared PC II is correlated with a relatively longer spinous dorsal-fin base and shorter anal- and soft dorsal-fin bases. A high score on PC I is correlated with a broadly connected branchiostegal membrane, a pigmentation pattern consisting of vertical bands, relatively more dorsal-fin spines and pectoral-fin rays, and fewer infraorbital pores and dorsal rays.

TABLE 1. VARIANCE LOADINGS OF MENSURAL CHARACTERS OF *Etheostoma* ON THE FIRST TWO SHEARED PRINCIPAL COMPONENTS.

Character	Shrd PCII	Shrd PCIII
Standard length	−.009	.074
Snout to occiput	−.028	.012
Occiput to spiny dorsal origin	−.061	.301
Spiny dorsal-fin base	.416	−.086
Soft dorsal-fin base	−.459	.181
Post. soft dorsal ray to hypural plate	.267	.034
Caudal peduncle depth	.128	−.231
Post. anal-fin ray to hypural plate	.155	.114
Anal-fin base	−.308	−.577
Pelvic insertion to anal-fin origin	−.014	.398
Snout to pelvic-fin insertion	−.018	.026
Snout to spiny dorsal origin	−.044	.112
Occiput to pelvic-fin insertion	.077	.077
Spiny dorsal origin to pelvic insertion	.078	.068
Soft dorsal origin to pelvic insertion	.144	.059
Spiny dorsal origin to anal origin	.076	.177
Soft dorsal origin to anal origin	.150	−.052
Soft dorsal origin to post. anal ray	−.182	−.043
Anal origin to post. soft dorsal ray	−.084	−.306
Post soft dorsal ray to post. anal ray	.135	−.238
Snout to jaw angle	−.269	.083
Post. anal ray to hypural plate	.170	.040
Post. soft dorsal ray to hypural plate	.246	.003
Head width at post. margin of orbit	−.133	−.163
Body width at pectoral fin insertion	−.077	.094
Head length	−.159	.100
Postorbital head length	−.164	.099
Orbit length	−.144	.173

Plotting sheared PC III vs PC I resulted in better separation of the purported hybrids from the *E. zonale* cluster (Fig. 3). Hybrid scores were lower on sheared PC III than scores of *E. zonale*. Individuals we identified as hybrids on the basis of pigmentation, shape of the branchiostegal membrane, and poorly formed palatine teeth had a shorter distance between the insertion of the pelvic fins and the anal-fin origin than the banded darter (Table 1). The distances from the nape to the dorsal-fin origin and from the anal-fin origin to the most posterior dorsal-fin spine were also relatively shorter in hybrids.

The determination of hybrid individuals in nature has traditionally been based on morphological intermediacy of the suspected hybrids (Hubbs, 1955). Individuals first hypothesized by us to be hybrids based on pigmentation pattern, branchiostegal membrane shape, and form of the palatine tooth patch were shown to be intermediate in overall morphology based on the analytical method of PCA (Figs. 2–3). That most

TABLE 2. VARIANCE LOADINGS ON THE FIRST PRINCIPAL COMPONENT FOR MERISTIC CHARACTERS.

Character	PCI
Lateral line scales	.013
Scales above lateral line	.050
Transverse scales, anal origin	.006
Transverse scales, soft dorsal origin	−.013
Least caudal peduncle scales	.025
Dorsal-fin spines	.108
Dorsal-fin rays	−.111
Anal-fin rays	−.071
Pectoral-fin rays	.109
Pelvic-fin rays	.031
Preoperculomandibular pores	−.098
Infraorbital pores	−.106
Branchiostegal rays	−.098
Breast squamation	−.057
Cheek squamation	−.019
Pigmentation pattern	.117
Shape of branchiostegal membrane	.118
Palatine tooth patch type	.117

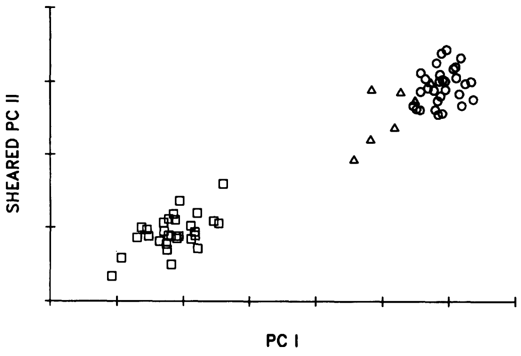

Fig. 2. Plot of individual scores on sheared PCII and PCI. Squares = *Etheostoma olmstedi,* circles = *E. zonale,* triangles = putative hybrids.

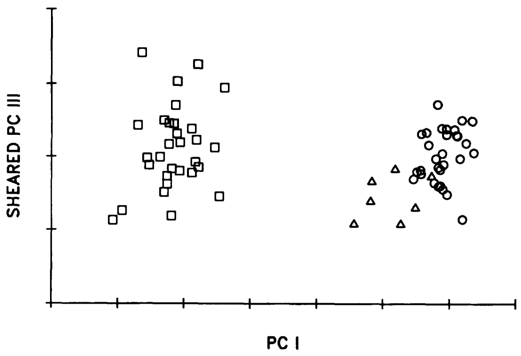

Fig. 3. Plot of individual scores on shared PCIII and PCI. Squares = *Etheostoma olmstedi,* circles = *E. zonale,* triangles = putative hybrids.

of these individuals lie outside the limits of variation of the purported parental species supports our hypothesis of hybrid origin.

Deviation from strict intermediacy in our sample may be due to the overall similarity of *E. olmstedi* and *E. zonale.* For example, there was overlap in 15 of the 18 meristic variables used in our analysis. All scale characters overlapped broadly, as did some of the fin element characters. Suzuki and Fukada (1973) found that intermediacy in salmonid hybrids was more likely for characters that were markedly different between parental species than for characters that were similar. Other studies of hybrid fishes have shown deviation from morphological intermediacy (Hubbs and Strawn, 1957; Ross and Cavender, 1981; Leary et al., 1983).

It is unlikely that the individuals described as hybrids represent extreme morphological variants of *E. zonale.* One of us (RLR) examined more than 650 collections of *E. zonale* from the upper Ohio River drainage from 1935–88. No specimens with the characters described above were observed in these collections.

The pattern of occurrence of hybrid individuals in the Susquehanna River drainage parallels the pattern of colonization of *E. zonale.* The first hybrids were collected from Pine Creek in 1971, the year and locality in which *E. zonale* was first collected. Subsequently, hybrids were collected in the lower Susquehanna River shortly after their first appearance in this area. However, because of our small sample size and the limited number of collections in this region in the mid-1970s, this observation offers little support for our hypothesis.

Due to the limited number of collections in the Susquehanna River drainage following the introduction of *E. zonale,* it is difficult to assess the extent to which hybridization has occurred. The number of unusual specimens obtained from the few available collections for this period indicates that hybridization may have occurred frequently.

An interesting feature of this hybridization event is the difference in reproductive behaviors between the two species. *Etheostoma zonale* is an egg attaching species (Trautman, 1957; Winn, 1958), while *E. olmstedi* possesses the more advanced egg-clustering behavior (Constantz, 1979). Hybridization may have taken place between these two species in one of three ways. Accidental contact of eggs and sperm from the two different species pairs mating in close proximity may have occurred. Another possibility is that *E. olmstedi* reverted to the more primitive behavior of *E. zonale.* Forbes and Richardson (1908) observed that *E. nigrum* (also an egg-clusterer) buried its eggs in an aquarium when suitable objects for attachment of eggs were unavailable. Page and Simon (1988) reported a similar observation for *E. punctulatum* spawned in an aquarium. The third possibility is that *E. zonale* actively participated with spawning *E. olmstedi* in the absence of conspecifics. The rarity of one species relative to another is a situation that is likely to occur following introductions (Hubbs and Miller, 1953; Hubbs, 1955). Because hybrids were collected from localities where *E. zonale* was rare, active participation of banded darters with mating *E. olmstedi* is the most likely explanation for hybridization, although the reversion of *E. olmstedi* to a more primitive reproductive mode cannot be ruled out. If accidental fertilization was responsible, hybrids would occur in regions where both *E. zonale* and *E. olmstedi* were abundant.

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