

# Intergeneric Spawning Between Captive Female Sacramento Perch (*Archoplites interruptus*) and Male Rock Bass (*Ambloplites rupestris*), Teleostei: Centrarchidae

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**ABSTRACT.**—The North American freshwater fish family Centrarchidae is well known for extensive natural hybridization, but there are no reports of voluntary spawning between genera. We document courtship and spawning in an aquarium between two separate pairs of a male Rock Bass (*Ambloplites rupestris*) and a female Sacramento Perch (*Archoplites interruptus*). One trial resulted in a low frequency of fertilized eggs, but these did not survive beyond the blastula stage. Fossil and molecular evidence suggests that these species have been isolated for at least 15 million years, so this spawning implies that courtship among species can persist longer during species divergence than previously appreciated.

## INTRODUCTION

Speciation can be defined as the gradual increase in reproductive isolation between two populations. Comparative studies have found that pre-mating isolation, hybrid sterility and hybrid inviability all accumulate steadily with the length of time separating pairs of populations (Coyne and Orr, 1989, 2004). For instance, hybrid embryo viability declines gradually in centrarchid fishes, with non-zero viability even for hybrids between divergent genera (Bolnick and Near, 2005; Hester, 1970; Tyus, 1973; West and Hester, 1966). Although hybrid viability persists over many millions of years, there are no documented cases of naturally occurring intergeneric spawning or hybrids within this family. The fact that hybrid viability is high among genetically and phenotypically distinctive species suggests that premating isolation is the dominant form of reproductive isolation among species. This implies that pre-mating isolation arises faster than hybrid inviability, a pattern also observed in a large number of other taxonomic groups (Coyne and Orr, 2004). In this paper we report evidence of incomplete pre-mating isolation among distantly related centrarchid species, based on observed spawning between female Sacramento Perch (*Archoplites interruptus*) and male Rock Bass (*Ambloplites rupestris*). This observation implies that pre-mating isolation can arise much more slowly than often believed.

The Sacramento Perch (*Archoplites interruptus*) is the sole representative of the freshwater fish family Centrarchidae native to western North America (west of the Rocky Mountains), being endemic to rivers of California's central valley (Moyle, 1976). The Rock Bass (*Ambloplites rupestris*) is native to the north eastern United States, ranging from Wisconsin to New York and south to Tennessee, sharing its range with numerous other centrarchid species (Lee *et al.*, 1981). The genera *Archoplites* and *Ambloplites* are sister genera, forming

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a monophyletic clade that is in turn related to *Pomoxis* and *Enneacanthus* (Near *et al.*, 2004). The monophyly of these two genera is strongly supported by molecular data from seven genes. The minimum estimated age of the clade is at least 15.5 million years before the present (mya), based on dating of the Clarkia Lake Beds in Latah Co, ID (Golenberg *et al.*, 1990) which contain fossil *Archoplites clarki* (Smith and Miller, 1985). Gap analysis (Marshall, 1990; Marshall, 1990; Strauss and Sadler, 1989) of a sequence of *Archoplites* fossils gives us 95% confidence that the clade arose not more than 18.7 mya (Near *et al.*, 2005). Five other fossil calibration points also predict that the *Archoplites/Ambloplites* divergence occurred approximately 15.5 million years ago (Near *et al.*, 2005). The Sacramento Perch and Rock Bass have thus been evolving independently since roughly the boundary between the early and mid Miocene. Since we generally expect pre-mating isolation to evolve much more quickly than post-mating isolation (Bolnick and Near, 2005; Coyne and Orr, 1997, 2004), any spawning between such divergent taxa would be noteworthy.

#### METHODS

Two mating trials were conducted in the fall of 2004. Experiments were not further replicated because a single observation of intergeneric spawning is sufficient to establish the fact that such spawning can occur (with the caveat that these trials are conducted in aquaria). In each case, a male Rock Bass and female Sacramento Perch were placed together in a 76 liter aquarium. The direction of this test cross was determined by availability of mature individuals. The aquarium was maintained at 27 C with a 250 watt aquarium heater, and aerated with a sponge filter. Photoperiod was 15:9 L:D. Two spawning substrates were placed in the tank: a 11.5 cm deep bucket with 3 cm of aquarium gravel, and a lengthwise section of PVC pipe (12.7 cm long, 7.6 cm diameter) covered with Spawn Tex™ and lightly sprinkled with gravel.

Prior to the experiments, fish were kept in a short-day photoperiod room (9:15 L:D). Each fish was transferred to separate tanks with 15:9 L:D photoperiod 2 to 3 mo before the breeding trial. Light was provided by a combination of natural light from two windows, and two 40-watt cool white fluorescent lights less than 1 meter from the water surface. The Sacramento Perch had been reared with conspecifics only, whereas the Rock Bass had been kept in a mixed group of Rock Bass and Sacramento Perch. Individual fish were chosen for the breeding trials based on the presence of a rounded belly indicating gravid status (female Sacramento Perch), and dark coloration and territoriality (male Rock Bass). The first trial used a 142 mm total length (TL) male Rock Bass and a 133 mm TL female Sacramento Perch. The Rock Bass was approximately 19 mo old, purchased from an aquarium supplier. The Sacramento Perch was 21 mo old and hatchery reared (F1 Sindicich Lagoon strain). The second trial used individuals of similar sizes (139 mm and 144 mm male and female TL respectively) and origin. Behavior was observed daily and the spawning of the first pair was recorded on film with a Sony VX3 Hi8 video camera. Eggs resulting from both trials were sampled with a dropper and observed under a compound microscope to determine fertilization rates, then maintained in a Petri dish at ~23 C to check viability.

#### RESULTS

The first pair of fish began interacting soon after being placed in the same tank. Both fish darkened in color and would face each other and lunge forward with gills flared. Each member of the pair would also display its body's broad side to the other, with fins erect and mouth open and swim in place. Both the male's dark color and aggressive behavior diminished over the following week, at which point a second reproductively mature Rock

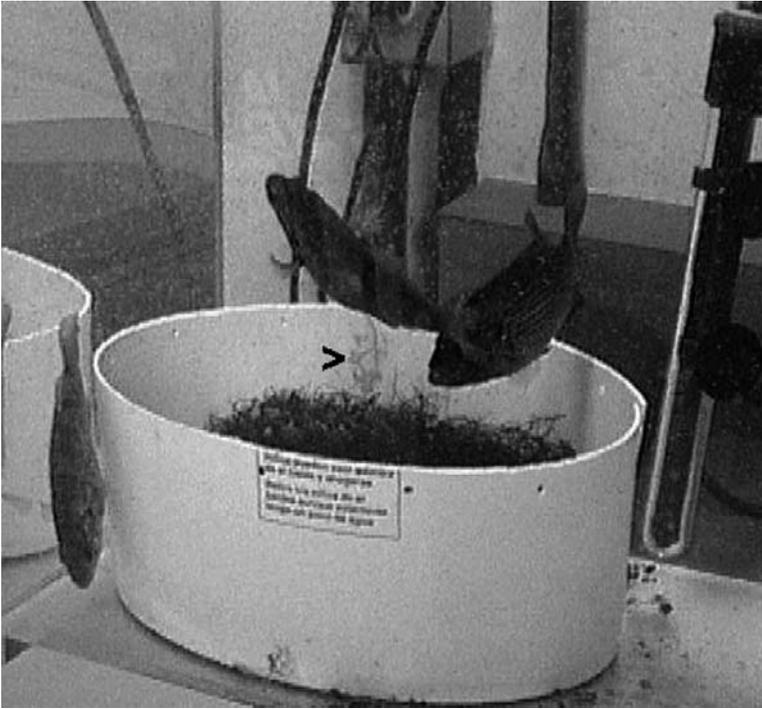


FIG. 1.—Female Sacramento Perch (*Archoplites interruptus*, center) laying eggs during spawning with a male Rock Bass (*Ambloplites rupestris*, right)

Bass male was introduced to the tank to stimulate the resident to new territorial activity. The resident male darkened further following this introduction and was very aggressive, and began to court the female Sacramento Perch the following morning.

During courtship, the male Rock Bass and female Sacramento Perch darkened and slowly swam in a circle over the substrate. The pair circled head-to-tail, but the female regularly changed directions. This caused the male Rock Bass to aggressively display, nip at the Perch's operculum and nudge her side to change the direction of movement. This behavior tended to last 5–10 min and was observed intermittently throughout the day. The following day, courtship resumed by 8:00 AM, with 10 to 15 min bouts of circling, and by 3:30 PM the courtship was almost continuous. At 4:28 PM, as the pair circled above the spawning area, the female Sacramento Perch angled her body to the side and expelled eggs from approximately 6 cm above the substrate (Fig. 1). The male Rock Bass inspected the eggs but made no attempt at fertilization. The pair would then resume circling until the female laid more eggs, for a total of fourteen clutches of eggs laid in approximately 11 min. The spawning and courtship sequence was recorded on a video camera, and is available from the authors on request.

After spawning the pair did not interact. On occasion the male was seen over the spawning area, but did not exhibit any territorial or fanning behavior. Eggs were sampled 4½ h after spawning and showed no signs of development, suggesting they had not been fertilized. All eggs were dead by the following day. The pair was kept together for 4 additional months, and did not spawn again despite intermittent courtship.

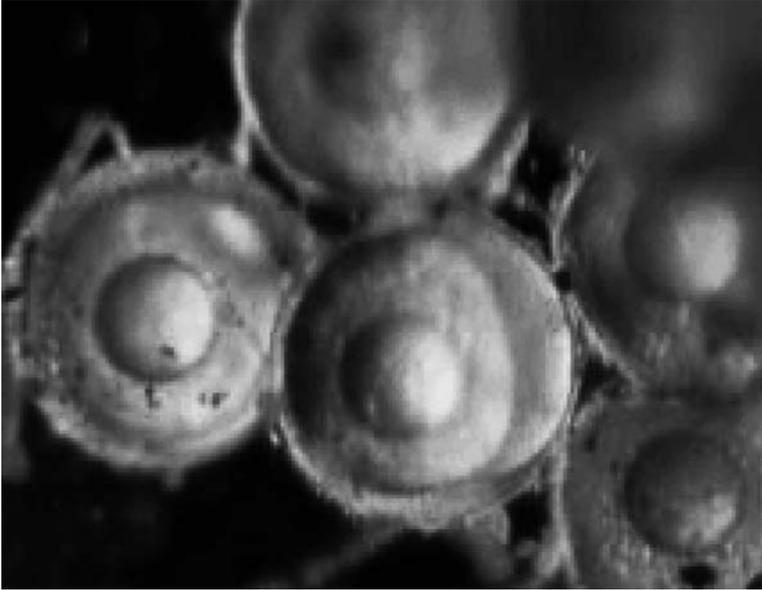


FIG. 2.—Developing embryos of hybrid *Archoplites interruptus* \* *Ambloplites rupestris*

The second mating trial also resulted in courtship, though this time the final spawning sequence was not directly observed, being inferred from the presence of fertilized eggs on the bottom of the tank 42 d after the trial began. Of approximately 150 eggs sampled, 8% ( $\pm 2$  SE) had reached the blastula stage (Fig. 2). None of the eggs developed past the blastula stage.

#### DISCUSSION

The observation of courtship behavior in both trials, and fertilized eggs in the second trial, demonstrates that pre-mating isolation between *Archoplites* and *Ambloplites* is incomplete. As far as we are aware, this is the first documentation of intergeneric spawning in the centrarchids. Previously reported crosses between the genera *Chaenobryttus* and *Lepomis* are now known to be intrageneric, since the former genus has since been shown to be a member of the genus *Lepomis* (Near *et al.*, 2004, 2005). While spawning has been known to occur between genera in other fish families (Sabaj *et al.*, 2000; Seehausen *et al.*, 1997), the genera in this study represent a very old split. That mate and courtship recognition could persist through 15 million years of independent evolution is remarkable, and suggests that pre-mating isolation can arise very slowly in some instances.

Extensive recent work on speciation and evolution has suggested that premating isolation plays a primary role in driving speciation in many organisms (Coyne and Orr, 2004). Within the Centrarchidae, recent analyses have confirmed that premating isolation may be the primary cause of speciation since post-mating isolation tends to evolve much more slowly than the waiting time between speciation events (Bolnick and Near, 2005). The observation provided here offers a counter-example in which post-mating isolation appears to be complete yet pre-mating isolation is not. This implies that populations,

diverging in allopatry, may retain some potential for courtship and mating for millions of years, in some cases accruing even more slowly than post-zygotic isolation. Such systems appear to be ideal for the operation of reproductive character displacement via reinforcement.

We speculate that *Archoplites* has not evolved rigorous mating isolation with other centrarchids because it has historically been allopatric with all other members of its family. As the sole member of Centrarchidae native to western North America, Sacramento Perch would not have experienced reinforcement of mating differences to avoid hybridization. Theory and extensive empirical evidence suggest that co-occurring closely related species frequently evolve divergent mating behaviors to avoid wasting gametes on producing hybrids (Coyne and Orr, 2004; Higgie *et al.*, 2000; Kirkpatrick, 2001). Isolated solitary endemic species may thus be particularly vulnerable to wasted reproductive effort and hybridization with introduced relatives.

*Acknowledgments.*—This manuscript benefited from comments by T. J. Near and P. C. Wainwright. We thank Contra Costa Mosquito & Vector Control District (CM) and the University of Texas at Austin (DIB) for support.

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SUBMITTED 7 NOVEMBER 2005

ACCEPTED 2 APRIL 2006