

Egg-mimicry as a mating strategy in the fantail darter, *Etheostoma flabellare*: females prefer males with eggs

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Summary. In some species of fishes with paternal care, females prefer to spawn with males already defending eggs. Such female preference appears to have resulted in adoption of unrelated eggs as a male mating strategy in several species. Page and Swofford (1984) proposed that such female preference may have also resulted in the evolution of male egg-mimics in several species of darters (Percidae); however, their hypothesis has not been tested. We examined female preference in the fantail darter (*Etheostoma flabellare*) and found that females preferred males with eggs over males without eggs, and males with egg-mimics over males without egg-mimics. Thus it appears that female preference for males already guarding eggs may have led to the evolution of specialized egg-mimicking morphology in males.

Introduction

Darwin's (1871) theory of sexual selection provides a powerful explanation for the evolution of male ornamentation and reproductive behavior in the animal kingdom. Of Darwin's two components of sexual selection, (1) male-male competition (intra-sexual selection) and (2) female choice (intersexual selection), female choice has generated the most research, discussion, and controversy (Bateson 1983; Bradbury and Andersson 1987). In teleost fishes with exclusive male parental care, the most common form of parental care in fishes (Blumer 1979, 1982; Gross and Shine 1981; Gross and Sargent 1985), the criteria known to affect female choice include male quality (Semler 1971; Down-

hower and Brown (1980), territory quality (Sargent 1982), and the presence of eggs in a male's nest (Ridley and Rechten 1981; Constanz 1985; Marconato and Bisazza 1986; Unger and Sargent 1988; Sikkell 1988). Female preference for males with eggs is particularly interesting, because it raises the question of how males without eggs compete for mates. Sexual selection may have produced two strategies in male fishes that exploit this female preference: adoption (Rohwer 1978) and egg-mimicry (Page and Swofford 1984; Page, in press). Although adoption has been documented in three species in which there is also evidence of female preference for males with eggs (van den Assem 1967; Ridley and Rechten 1981; Constanz 1985; Unger and Sargent 1988), egg-mimicry is still undocumented as a mating strategy. Page and Swofford (1984) hypothesized that egg-mimicry in males of several species of darters (genus: *Etheostoma*; subgenus: *Catonotus*) evolved in response to female preference for males with eggs. We examined the putative phenomenon of male egg-mimicry in the most common of these species, the fantail darter, *Etheostoma flabellare*.

Fantail darters are small, stream-dwelling, percid fish that range widely in eastern North America (Kuehne and Barbour 1983; Page 1983). During the spring, males excavate nests beneath flat rocks, defend small territories, and mate with one to many females. Only males care for the developing eggs, which are attached by females to the ceiling of the nest (Winn 1958).

The hypothesized egg-mimicking structures of the male fantail darter are located on the distal margin of the first dorsal fin (Page and Swofford 1984; Page, in press; Fig. 1A). Each of the seven to eight dorsal spines is tipped with a fleshy knob, which is trailed posteriorly by a pigmented, semi-circular ocellus in the fin membrane. The ocelli

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are orange, bordered in black, and surrounded by colorless, transparent membrane. When the distal edge of a male's first dorsal fin touches the ceiling of his nest, as happens during courtship and nest tending, it closely resembles a row of eggs in location, color, and shape (Fig. 1A).

We examined Page and Swofford's (1984) egg-mimicry hypothesis in the fantail darter and predicted the following: (1) egg-mimics would be larger and more developed in males than in females, (2) females would prefer to spawn with males with eggs over males without eggs, and (3) females would prefer to spawn with males with egg-mimics over males without egg-mimics.

Methods

We observed the breeding behavior of fantail darters from Shelby Branch, a second order spring-fed stream in central Kentucky, from late March to early June 1988. Fish were maintained in the laboratory at 18°C under the ambient photoperiod and were fed twice daily on a diet of amphipods, tubifex worms, cladocerans, and frozen brine shrimp. Female choice experiments were conducted in replicate 150-liter aquaria, each containing two courting males and two artificial nest sites. Gravid females were placed in each aquarium and a female mating preference was recorded only for the first spawning event per aquarium. Female preference was analyzed by comparing the distribution of choices against the one-tailed binomial probability of this distribution occurring at random when $P=0.5$ (Sokal and Rohlf 1981). Males were individually color-marked with 10-microliter subcutaneous injections of dilute acrylic paint (Liquitex), using 25G syringes (e.g., Thresher and Gronell 1978; Unger 1983). The paints were diluted approximately 1:4 in deionized tap water. Artificial nest sites consisted of 15 cm × 15 cm clay tiles laid flat over small depressions in the substratum. Males readily nested under these tiles, both in the field and laboratory, and excavated any excess substrate from beneath the tiles.

1. *Egg-mimic morphology.* Egg-mimic diameters were measured on 24 males and 23 females, all in breeding condition. Egg-mimics were measured to the nearest 0.01 mm with an ocular micrometer, under 60× magnification. Prior to measurement, fish were anesthetized with 3-aminobenzoic acid ethyl ester (MS-222). We defined egg-mimic diameter as the longest distance across the fleshy knob and pigmented ocellus and plotted mean egg-mimic diameter versus standard length (SL; i.e., the tip of the snout to the posterior end of the vertebral column) for each fish. Egg-mimic sizes were also compared against the average egg diameter, which was obtained by measuring 50 eggs randomly chosen from different nests.

2. *Egg preference.* To test whether females prefer males with eggs, we allowed females to choose between a male with eggs and a male without eggs in laboratory aquaria. There were two treatments. First, we examined female preference for a male with eggs versus a male whose eggs had been removed; a female's preference was scored once she spawned. The nests of males with eggs in this manipulation contained approximately 160 eggs, which represents roughly four times the average female clutch. Second, we examined female preference for a male without eggs versus a male to whose nest we assigned eggs. Preference in this treatment was scored as the spatial distribution of females between the two males.

In the egg-removal treatment, a pair of males, their respective nest-sites, and three gravid females were introduced into

each of eight aquaria. To obtain males with eggs, we placed clay nest-tiles in Shelby Branch and after several days, egg-guarding males and their nest-tiles were collected and taken to the laboratory. Pairs of males with eggs were matched as closely as possible in length and weight, and eggs of one randomly-chosen male within each pair were removed. In the egg-addition treatment, we examined the spatial distribution of females between two nest-tiles, one with and one without eggs. We placed two males, two nest-tiles, and eight females into each of five aquaria. After the males were defending nest-tiles, one randomly-chosen nest-tile in each aquarium was replaced with one containing eggs. The number of females under each nest-tile was then recorded once per minute for 6 min. The nest-tiles with eggs were then reassigned to the males that initially were without eggs, and the trial was repeated. Because neither male within an aquarium sired the eggs in this experiment, we protected the eggs from potential predation with 9 cm water-filled petri dishes; petri dishes were also placed under the nest-tiles without eggs.

3. *Egg-mimic preference.* To test the hypothesis that females prefer males with egg-mimics, we allowed females to choose between males with and without egg-mimics. There were two treatments. First, we compared female preference for a male with egg-mimics versus a male whose egg-mimics had been removed; preference was scored when a female spawned. Second, we examined the spatial distribution of females between two passive male dummies, one with and one without egg-mimics.

In the egg-mimic removal treatment, a pair of males, two nest-tiles, and three gravid females were introduced into each of 17 aquaria. Courting males were collected from Shelby Branch and paired to minimize size differences. We removed egg-mimics by clipping the distal 2 mm of the first dorsal fin with fine scissors. This procedure seemed to have little effect on male behavior, and males usually resumed courtship within 10 min of egg-mimic removal. In addition, males in Shelby Branch often had missing egg-mimics, apparently as a result of agonistic interactions or attempted predation. In the first 3 replicates, we controlled for any effects of egg-mimic removal on male behavior by removing the egg-mimics from the larger or more dominant male. In the remaining 14 replicates, the males whose egg-mimics were removed were chosen randomly. Here, we controlled for effects of egg-mimic removal by clipping a similar portion from the second dorsal fin (which serves no mimicking function) of the control males.

A treatment using dummies was conducted to control for the possibility that males without egg-mimics might court less intensely than males with egg-mimics. Two passive male dummies, one with and one without egg-mimics, two nest-tiles, and eight females were introduced into each of five aquaria. We formed 67 mm SL dummies from brown modeling clay. The dummies differed only in the color of their first dorsal fins, which were constructed from 3 mm × 19 mm strips of colorless, transparent acetate. Orange dots 2.1 mm in diameter were painted on one dummy's dorsal fin (2.1 mm is the egg-mimic size predicted by the regression equation of egg-mimic diameter on male standard length). The other dummy's dorsal fin was unpainted. Within each aquarium, each dummy was placed beneath a nest-tile with the distal margin of its dorsal fin flush with the ceiling of the nest to imitate the silhouette of a courting male. The number of females beneath each nest-tile was recorded once per minute for 6 min. Positions of the dummies were then switched and the trial repeated.

Results

1. *Egg-mimic morphology.* Structures resembling eggs were present on the first dorsal fins in both

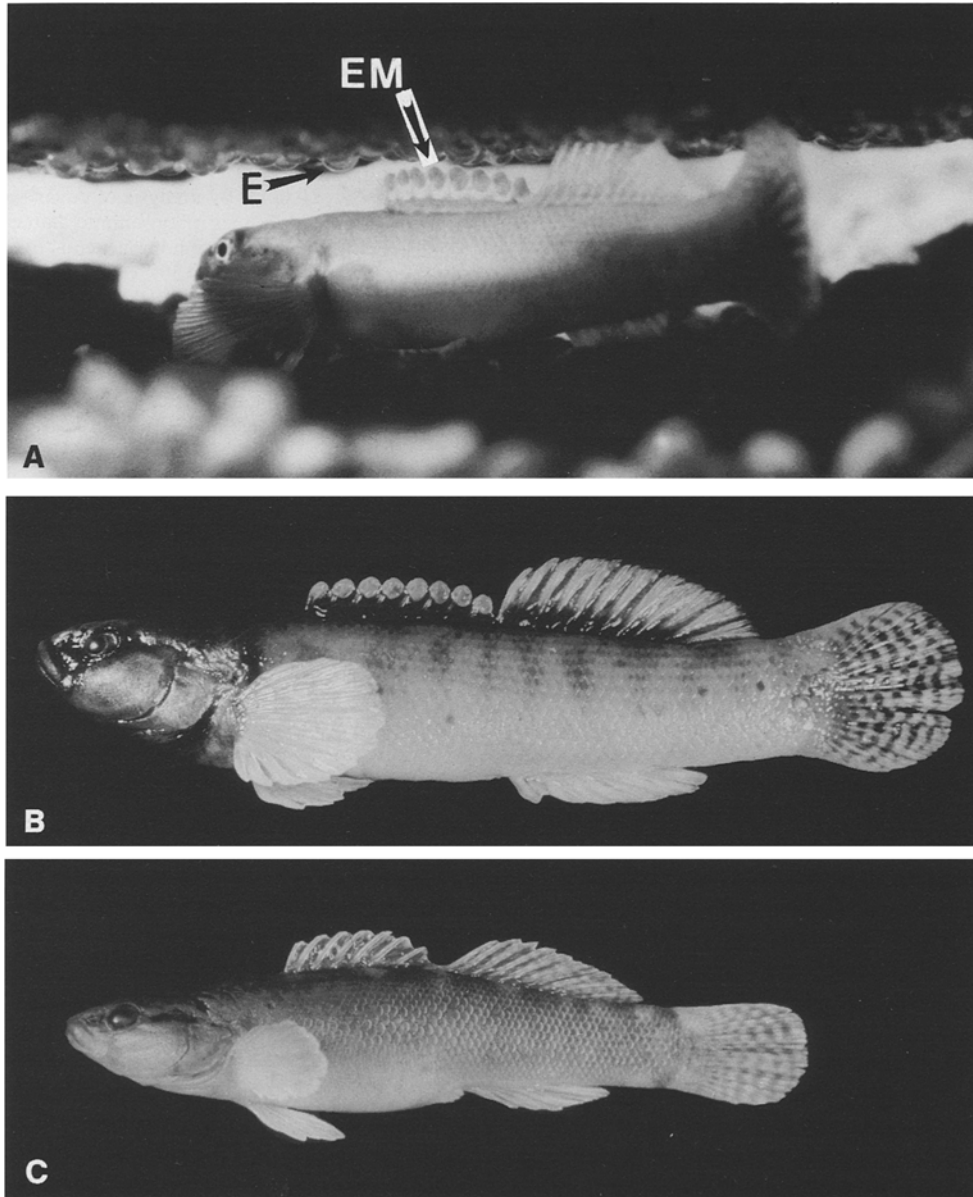


Fig. 1. (A) A breeding male fantail darter defending eggs in his nest. The egg-mimics (EM) are located on the distal margin of his first dorsal fin, which appears just below the eggs (E) on the ceiling of the nest cavity. (B, C) Close-ups of breeding male (B) and female (C) fantail darters, illustrating sexual dimorphism in body size and egg-mimic development. Photographs (B) and (C) by Roger W. Barbour; photograph (B) is reproduced with permission of the University Press of Kentucky

sexes; however, egg-mimics were larger and much more fully developed in males (Fig. 1B). Female egg-mimics lacked fleshy knobs, were less brightly colored, and were smaller in diameter, even after adjustment for the smaller female body size (Fig. 1C, Fig. 2). Moreover, egg-mimic size was positively correlated with body size, and only approached the size of eggs in the largest males (Fig. 2). Newly fertilized eggs averaged 2.56 mm in diameter ($s=0.18$, $N=50$), range: 2.30–3.03 mm), which included the orange yolk body and the surrounding perivitelline space.

However, because the egg membrane and perivitelline space are transparent and nearly invisible, the yolk body may be a better measure in comparisons of egg-mimic size to egg size. The average diameter of the yolk bodies was $2.09 \text{ mm} \pm 0.17$. According to the regression equation of male egg-mimic diameter versus standard length, 64 mm males are expected to have egg-mimics the size of the average yolk body.

2. *Egg preference.* In the egg-removal treatment, seven out of eight females spawned with the male whose nest-tile contained eggs ($P=0.035$, one-

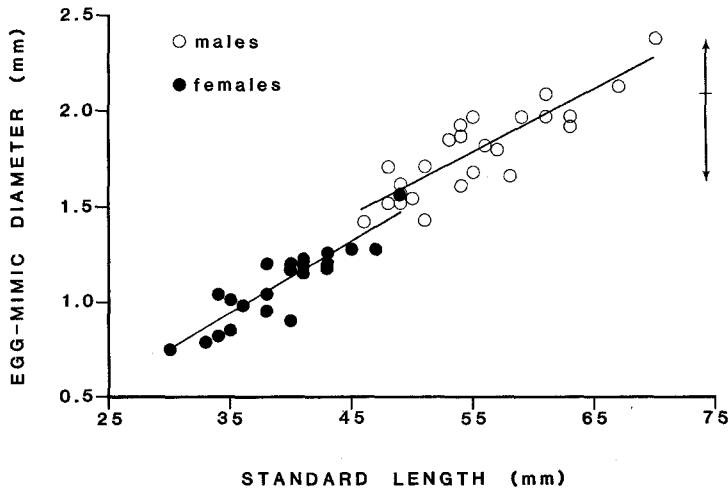


Fig. 2. Egg-mimic diameter versus standard length (SL). Males were larger than females (males: mean SL = 55.5 mm, $s = 6.28$, $N = 24$; females; mean SL = 39.1 mm, $s = 4.66$, $N = 24$; $t = 10.274$, $P < 0.001$). Egg-mimic diameter increased with SL in both sexes (males: $Y = 0.03X - 0.031$, $N = 24$, $t = 8.156$, $P < 0.001$; females: $Y = 0.037X - 0.372$, $N = 23$, $t = 8.798$, $P < 0.001$). By analysis of covariance, the two regression lines of egg-mimic diameter versus standard length were homogeneous in slope ($F = 0.538$, $df = 1, 43$, $P > 0.50$), but differed in elevation ($F = 6.036$, $df = 1, 44$, $P < 0.025$). Males had larger adjusted mean egg-mimics than females. At the right edge of the graph is the observed range (double headed arrow) and mean (cross hatch) for egg yolk diameter (mean = 2.09 mm; range = 1.64 mm–2.38 mm; $N = 50$)

Table 1. The results of the two egg versus no-egg treatments and the one-tailed binomial probabilities for female preference for males with eggs. *A.* Egg removal: In 7 out of 8 replicates, females spawned with the male with eggs. *B.* Egg addition: In 5 out of 5 replicates, females were observed more frequently beneath the nest-tile with eggs. The summed observations of females beneath nest-tiles with and without eggs are given for each replicate

Egg preference experiment

Treatment	Eggs	No eggs	<i>P</i>
<i>A.</i> Egg removal: Preference = Spawning	7	1	0.035
<i>B.</i> Egg addition: Preference = Spatial association	5	0	0.031
Aquarium no.			
1	30	8	
2	19	3	
3	24	8	
4	20	4	
5	18	5	

tailed binomial probability, Table 1 A). Males with and without eggs did not differ significantly in SL or weight (paired *t*-test; SL: $t = 0.084$, $df = 7$, $P > 0.90$; weight: $t = 0.423$, $df = 7$, $P > 0.50$). Female egg predation was observed frequently during this treatment.

In the egg-addition treatment, females actively moved between nest-tiles; however, in all five aquaria they were observed more frequently under the nest-tiles with eggs ($P = 0.031$, one-tailed binomial probability, Table 1 B).

3. Egg-mimic preference. In the egg-mimic removal treatment, females tended to spawn with the male with egg-mimics. In the first 3 replicates, all spawnings went to the males with egg-mimics; in the

Table 2. The results of the two egg-mimic versus no egg-mimic treatments and the one-tailed binomial probabilities for female preference for males with egg-mimics. *A.* Egg-mimic removal: In 13 out of 17 replicates, females spawned with males with egg-mimics. *B.* Dummy: In 5 out of 5 replicates, females were observed more frequently next to the dummy with egg-mimics. The summed observations of females beneath nest-tiles with dummies with and without egg-mimics are given for each replicate

Egg-mimic preference experiment

Treatment	Egg-mimics	No egg-mimics	<i>P</i>
<i>A.</i> Egg-mimic removal Preference = Spawning	13	4	0.025
<i>B.</i> Dummy: Preference = Spatial association	5	0	0.031
Aquarium no.			
1	20	5	
2	17	6	
3	21	6	
4	17	11	
5	15	5	

remaining 14 replicates, 10 spawnings went to the males with egg-mimics. The distribution of spawnings between these two groups appeared to be statistically homogeneous ($G = 0.101$, $df = 1$, $P > 0.50$, Yates correction applied); therefore, we analyzed the distribution of spawnings for the pooled data. Thirteen out of 17 total spawnings occurred with the male with egg-mimics ($P = 0.025$; one-tailed binomial probability, Table 2 A). Males with and without egg-mimics did not differ significantly in SL or weight (paired *t*-test; SL: $t = 1.461$, $df = 16$, $P > 0.10$; weight: $t = 0.280$, $df = 16$, $P > 0.50$). Again, egg predation by females was observed frequently in this treatment.

In the dummy treatment, females tended to as-

sociate with the dummy with egg-mimics. In all five aquaria, females were observed more frequently beneath the nest-tiles with the dummy with egg-mimics ($P=0.031$, one-tailed binomial probability, Table 2B). Females were observed nipping at the dummy's egg-mimics on several occasions.

Discussion

These experiments support each of our predictions: (1) egg-mimics were more highly developed in males; (2) females preferred males with eggs over males without eggs; and (3) females preferred males with egg-mimics over males without egg-mimics. Thus, our results provide circumstantial evidence for Page and Swofford's (1984) hypothesis; that is, it appears that the putative male egg-mimics may have evolved as a mating strategy, via intersexual selection, in response to female preference for males with eggs. Male-male competition appeared unimportant in our experiments; we observed little interaction between males, whereas females actively moved between nest tiles. Thus, our results imply that female choice played a major role in the evolution of male egg-mimics. However, our investigation was limited to one population and one breeding season. Future research on the egg-mimicry phenomenon, both within and among populations, will help determine the generality of our findings.

It now becomes interesting to investigate the evolutionary origins of male egg-mimics. Fleishy knobs on the tips of fin spines are known in several species of fishes with paternal care, and in darters are hypothesized to function in preventing accidental puncture of the eggs during routine nest tending behavior (Page and Swofford 1984; Page, in press). In the fantail darter, these knobs are relatively large and have taken on the orange coloration of eggs. Furthermore, most darter species have characteristic patterns of stripes on the dorsal fins, which are especially pronounced in males (Kuehne and Barbour 1983; Page 1983). In the fantail darter it appears that one of these stripes may have been modified over evolutionary time to be a row of discrete orange ocelli (Fig. 1). Taken together, the fleshy knobs and ocelli closely resemble the eggs of this species and appear to have converged on this resemblance through female preference for males with eggs. We suggest that the close resemblance between egg-mimics and eggs, combined with our findings of female preference for males with eggs and for males with egg-mimics, suggests that male egg-mimics do not function simply as species recognition cues, or as mere secondary sex-

ual characters. Future experiments to further test Page and Swofford's (1984) hypothesis will include a detailed behavioral analysis of how male egg-mimics are used during courtship.

Assuming that Page and Swofford's (1984) egg-mimicry hypothesis is correct, we are still left with the question as to why females prefer males with eggs, and males with egg-mimics? There is little empirical evidence on an adaptive basis for female preference for males with eggs in fishes with paternal care. However, in the fathead minnow *Pimephales promelas*, paternal care and egg survival both increase with clutch size (Sargent 1988), and females prefer to spawn with males with eggs (Unger and Sargent 1988). A similar mechanism may operate in the fantail darter. Alternatively, females may associate with males with eggs because eggs are a source of food. In some fishes with paternal care, females are voracious predators on conspecific eggs (e.g., Kynard 1978; Unger 1983). We observed frequent female egg predation and even female nipping of male egg-mimics. Egg predation by female fantail darters may result in their spending a disproportionate amount of time near nests with eggs, and thus they may increase their chances of spawning in such nests. Male egg-mimics may exploit this female motivation. More research is needed to determine the potential roles of female egg cannibalism in the evolution of female preference for males with eggs and in the evolution of male egg-mimics.

Female preference for males with egg-mimics might also result in higher egg survival. We found that egg-mimic size increased with male body size, and only approached the size of real eggs on the largest males in the population (Fig. 2). Egg-mimic size is probably constrained by the distance between a male's dorsal spines, and thus by male body size. Male body size and egg survival are positively correlated in other fishes with paternal care (e.g., Downhower and Brown 1980; Gross 1980). If this pattern were also true for male fantail darters, then females might assess the quality of a male's parental care through the size of his egg-mimics. The testing of these hypotheses will be the subject of future research.

Egg-mimicry has been documented in one other family of fishes, the Cichlidae, where they may serve a different function from that proposed for fantail darters. In some maternal mouth-brooding cichlids, male egg-mimics appear to aid in fertilization and apparently evolved in response to female mouthbrooding behavior (Wickler 1962; Mrowka 1987). After spawning, the female takes the eggs into her mouth before fertilization by the male.

The male moves to where the eggs were, releases sperm into the water, and flares his anal fin, exposing several egg-mimicking spots. The female attempts to take these egg-mimics into her mouth and has her eggs fertilized in the process (Wickler 1962; Mrowka 1987). Contrary to Wickler (1962) and Mrowka (1987), Hert (1989) found no evidence that male egg-mimics function in enhancing fertilization in the maternal mouthbrooder, *Astatotilapia elegans*. Hert did find, however, that females preferred males with egg-mimics over males whose egg-mimics had been removed. Thus, the maintenance of egg-mimics in this species appears to be due to female choice. Whether or not cichlid egg-mimics originally evolved as an aid to fertilization and secondarily took on the role of intraspecific sexual advertisement is an open question.

In the fantail darter, male egg-mimics clearly do not aid in fertilization; however, they do seem to function in mate attraction. Thus, in fantail darters it appears that female preference for males already guarding eggs may have led to the evolutionary specialization of male tissues to resemble eggs.

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