

## A test for preference of association in a color polymorphic poeciliid fish: laboratory study

Charles F. Baer,<sup>1,2</sup> Marc Dantzker<sup>1,3</sup> & Michael J. Ryan<sup>1</sup>

<sup>1</sup> Department of Zoology, The University of Texas, Austin, TX 78712, U.S.A.

<sup>2</sup> Current address: Department of Biological Sciences, Florida State University, Tallahassee, FL 32306, U.S.A.

<sup>3</sup> Current address: Department of Zoology, Duke University, Durham, NC 27706, U.S.A.

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### Synopsis

The ecological and evolutionary forces maintaining genetic polymorphism within populations is of continuing interest to evolutionary biologists. Male pygmy swordtails, *Xiphophorus pygmaeus*, are polymorphic at a Y-linked locus controlling body color. Fish with the + and cp alleles have blue bodies; those with the con allele have bright gold bodies. Male and female fish were tested in the laboratory to determine if there were any preferences for association with groups of male fish based on color. Single test fish were presented with two groups of males of different phenotypes, and times in which the test fish spent in proximity to each group were recorded. Males of different phenotypes showed no differences in preference, nor did phenotype of the males in the groups affect the behavior of the test fish. Females also showed no preference for males of a particular phenotype. Finally, female test fish showed no preference for groups containing a male with the 'rare' phenotype compared to a group containing all males of the same phenotype.

### Introduction

The maintenance of genetic polymorphisms, especially those with apparent phenotypic consequences, remains one of the most important and interesting issues in evolutionary biology. In general, there are a number of selective phenomena that can serve to maintain genetic polymorphism (Felsenstein 1976, Hedrick 1986). Balancing natural and sexual selection, for example, maintains polymorphism at a Y-linked size-controlling locus in the swordtail *Xiphophorus nigrensis* (Ryan et al. 1992).

*Xiphophorus pygmaeus* Hubbs and Gordon (Pisces: Poeciliidae), the slender pygmy swordtail, provides an excellent opportunity to investigate the potential role of ecological and behavioral forces in

such maintenance. This small (20–38 mm standard length) livebearing fish is found in several tributaries of the Rio Panucó in eastern Mexico. Mature males are polymorphic at a single Y-linked (and therefore functionally haploid) locus that controls body color. Fish with the + allele are grayish blue, with bright blue sides. Those with the cp allele show the same body colors as fish with the + allele, but have a yellow caudal fin. Males with the con allele have bright yellow bodies as well as caudal fins (Kallman 1989). Females are a duller blue-gray with a yellow tint on the dorsal surface. Observations over a seven-year period have shown that the frequency of the con allele in a particular population varies from about 0.25 to 0.4, with an average of about 0.33. Fish with the + and cp alleles were ini-

tially lumped into the 'ecotype' blue, due to their phenotypic similarity, but approximately 25% of blue males have the cp allele (unpublished data). Some males with either the + or the cp allele have a gold tint on the dorsal surface (similar to female pigmentation); this trait is not allelic at the locus in question, and the genetic basis of this trait, if any, is unknown (MJR & M. Morris unpublished data).

In this study we performed laboratory experiments designed to detect phenotype-based differences in preferences of association in both male and female fish. Differences in preferences of association could provide evidence for several different ecological and/or evolutionary phenomena. A difference in preferences of association between phenotypes of male fish could provide evidence for differences in schooling behavior. A preference of association in females could indicate the presence of female mating preference (e.g. McPhail 1969, Endler 1983, Ryan et al. 1990). A preference in fish of both sexes to associate with fish of a given phenotype could indicate the presence of a sensory bias, i.e., fish may be more capable of visually detecting fish of one color than of another. Ecologically relevant visual biases have been shown to exist in fish (Endler 1991).

## Methods and materials

A series of experiments was designed to detect possible preferences of association in fish of a given phenotype and/or sex. The standard procedure was to place a single fish (called a 'test fish') in the center compartment of a partitioned 89.9 cm long  $\times$  40.6 cm wide  $\times$  45.7 cm deep glass tank filled to 30.5 cm deep, and to place groups of five male fish of designated phenotypes in the side compartments. Each side compartment was 17.8 cm long, and partitions consisted of solid Plexiglas sheets attached to the tank with a continuous strip of silicone caulk. The test fish was placed in the center compartment, with blinds placed between the center compartment and the side compartments. After an approximately 2 min period of acclimation, the blinds were lifted, and the behavior of the test fish was recorded on videotape for 10 min. After 10 min,

the blinds were lowered, and the groups of fish were switched, to control for potential side biases. The above procedure was then repeated for 10 min. The center compartment of the tank was marked off into three 17.8 cm long sections. The amount of time the test fish spent in each section was determined from a counter on the videotape. Time spent in the section nearest a given group was interpreted as a preference on the part of the test fish to associate with that group. Time spent in the center section was interpreted as no preference. In all experiments, half of the trials began with the group of a given phenotype on one side of the tank and the other half of the trials began with that phenotype on the other side of the tank. The order was randomized in all experiments.

The background consisted either of the inside of the laboratory as seen through a transparent fish tank, or of an enlarged photograph taken approximately 20 cm underwater in the Rio Huichihuayan, the home site of the fish tested. Background was kept constant within an individual experiment. Lighting consisted of fluorescent laboratory lights combined with natural daylight entering from windows behind the tank. Daylight varied with cloud cover, time of day, and season. All experiments were performed between 13:00 and 17:00 h. Tanks were not artificially temperature controlled, but laboratory ambient temperature remained a relatively constant  $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

### *Male test fish with groups of males*

Groups of 5 con and 5 blue males were chosen haphazardly from pools of 9 fish of each phenotype. As noted in the introduction, the category 'blue' consisted of both + and cp fish. Fish were size-matched to  $\pm 1.5$  mm standard length within the pools of 9 so that each pool had approximately the same mean standard length and standard deviation. Size disparities may have occurred within individual trials. Upon completion of a trial, fish in the groups of 5 were returned to the pool, and 5 fish were chosen from the 9 in the same manner for the next trial. Two fish died during the experiment and were replaced with males approximately equal in length ( $\pm$

1 mm). Ideally, the 5 fish in an experimental group should have been discarded and 5 new fish chosen for the next trial. However, we did not have sufficient fish to do this, and we believe that our method was the best compromise available. The fish that constituted the pools were kept in the same holding tank, so all fish should have had approximately the same environmental influences. Test fish were kept in two holding tanks, with blue and con fish mixed together. All fish were wild caught in April 1992. The background for this experiment was the underwater photograph. Twenty four different test fish of each phenotype were tested.

#### *Female test fish with groups of males*

The procedure was the same as in the previous experiment. We performed this experiment twice, in December 1991, and August 1992. In the December experiment, the pool consisted of 12 males of each phenotype. The distribution of sizes ranged from 20.0 to 24.0 mm standard length, but the mean lengths and standard deviations were approximately equal. In this experiment, the background consisted of the laboratory as seen through the tank. Again, the pool males were kept in the same holding tank. All female test fish were kept in the same holding tank, and were kept segregated from males. All males were wild-caught in October 1991. Females were wild caught in either June or October 1991.

In August we repeated this experiment with the underwater photograph background. This time the pool of males consisted of the same pool used in the male choice experiment described above. Females were wild caught in April 1992.

Subsequent to the December experiment, the experiment was repeated with the same female test fish, but with schools of + and cp males. Because our procedure involved pooling + and cp males into the 'ecotype' blue, we wanted to determine if test fish discriminated between the two phenotypes. Preliminary tests with male test fish indicated that test fish did not discriminate between the two phenotypes (Baer 1992). In this experiment, the pool consisted of 11 males of each phenotype, size matched as be-

fore, with a distribution from 21.5 to 26.0 mm. This experiment was performed in February 1992, and the background was again the laboratory as seen through the tank.

#### *Female test fish with rare males*

This experiment was designed to test the hypothesis that female test fish prefer to associate with a group of males that contains a single male of a 'rare' phenotype than with a group composed of all males of the same phenotype (e.g. Farr 1977). The procedure was essentially the same as above, except that one group was either all blue or all con, and the other group consisted of four blue or con and one male of the other phenotype. Test females were the same fish used in the December female choice experiment. In the rare blue experiment, the pool consisted of 6 blue and 16 con fish, from 20.5 to 22.5 mm standard length. In the rare con experiment, the pool consisted of 6 con and 16 blue fish, from 20.0 to 23.0 mm standard length, distributed as before. These experiments were performed with the same females used in the December female choice experiments, and the background consisted of the laboratory as seen through the tank. All pool males were kept in the same holding tank, as were the test females.

#### *Statistical analyses*

All pairwise tests of preference were analyzed with two-tailed paired Student's t-tests. In all experiments the null hypothesis is homogeneity of means between the groups tested.

### **Results**

Neither con males nor blue males showed any significant preference for groups of a particular phenotype (Table 1; blue males, two-tailed paired t-test,  $t = 0.139$ ,  $df = 23$ ,  $p > 0.89$ ; con males,  $t = 0.491$ ,  $df = 23$ ,  $p > 0.62$ ). Average times spent with con fish did not differ significantly between con and blue test

**Table 1.** Mean times of association for male test fish. Times are given as proportions,  $\pm$  SD. See text for details. Times of association of test fish of a given phenotype were tested against the null hypothesis of equal means with two-tailed paired Student's *t*-tests. In neither case did mean time of association differ from expected under the null hypothesis (blue,  $t = 0.139$ ,  $df = 23$ ,  $p > 0.89$ ; con,  $t = 0.491$ ,  $df = 23$ ,  $p > 0.62$ ). Time of association of blue and con test fish with the con group was tested against the null hypothesis of equal means with an unpaired *t*-test. Mean times of association did not differ from expected under the null hypothesis ( $t = 0.107$ ,  $df = 46$ ,  $p > 0.91$ ).

| Test fish phenotype | n  | Phenotype of group |                   |
|---------------------|----|--------------------|-------------------|
|                     |    | blue               | con               |
| blue                | 24 | 0.481 $\pm$ 0.252  | 0.467 $\pm$ 0.236 |
| con                 | 24 | 0.494 $\pm$ 0.159  | 0.461 $\pm$ 0.171 |

fish (Table 1; unpaired *t*-test,  $t = 0.107$ ,  $df = 46$ ,  $p > 0.91$ ).

Female test fish showed no significant preference for groups of males of either the blue or the con phenotype. This result held in both December and August, against both types of backgrounds (Table 2; December, two-tailed paired *t*-test,  $t = 1.414$ ,  $df = 23$ ,  $p > 0.17$ ; August,  $t = 0.363$ ,  $df = 16$ ,  $p > 0.72$ ). Average times spent with con fish did not differ significantly between December and August (Table 2; unpaired *t*-test,  $t = 1.14$ ,  $df = 39$ ,  $p > 0.26$ ). Neither did female test fish show a significant preference to associate with groups of blue males with either the + or the cp allele (two-tailed paired *t*-test,  $t = 1.115$ ,  $df = 25$ ,  $p > 0.27$ ).

Finally, female test fish showed no significant preference for rare males of either phenotype (Table 3; rare blue male, two-tailed paired *t*-test,  $t = 1.219$ ,  $df = 25$ ,  $p > 0.23$ ; rare con male,  $t = 0.473$ ,  $df = 25$ ,  $p > 0.64$ ). Average times spent with rare males did not differ significantly between rare con and rare blue groups (Table 3; unpaired *t*-test,  $t = 1.558$ ,  $df = 50$ ,  $p > 0.12$ ).

## Discussion

Our laboratory experiments showed unambiguously the lack of strong preferences of association in both males and females under our laboratory con-

ditions. Previous experiments done under the same lighting conditions designed to test female preferences for individual males of different phenotypes indicated a trend suggesting that female *X. pygmaeus* may prefer to associate with blue males, but these experiments were inconclusive (MJR & M. Morris unpublished data). Neither of our female choice experiments indicated the presence of a strong preference. It is possible that there is a preference for some male behavior correlated with color phenotype that is not apparent when males are put in a group (J. Ellingson personal communication), but it seems clear that females do not have a strong preference for color per se. Females did not discern between blue males with the + allele and those with the cp allele when males were matched for size, as expected. In addition, females did not prefer to associate with a group of males containing a male with a rare phenotype, although in both cases test fish spent more time with the group with the most blue males in it.

*X. pygmaeus* is typically found in water less than a meter deep in a (usually) clear, spring-fed river (CFB personal observation), so the light regime in our test tank was probably qualitatively similar to that found in the river, at least in the dry season. *X. pygmaeus* is found in habitats with considerable aquatic vegetation, however, and it is possible that the

**Table 2.** Mean times of association for female test fish. Times are given as proportions,  $\pm$  SD. See text for details. Times of association of females with groups of a given phenotype within a trial were tested against the null hypothesis of equal means with two-tailed paired Student's *t*-tests. In neither case did mean time of association differ from expected under the null hypothesis (December,  $t = 1.414$ ,  $df = 23$ ,  $p > 0.17$ ; August,  $t = 0.363$ ,  $df = 16$ ,  $p > 0.72$ ). Times of association of test fish with con fish between December and August were tested against the null hypothesis of equal means with an unpaired *t*-test. Time of association did not differ significantly from expected under the null hypothesis ( $t = 1.14$ ,  $df = 39$ ,  $p > 0.26$ ).

| Trial (month)                       | n  | Phenotype of group |                   |
|-------------------------------------|----|--------------------|-------------------|
|                                     |    | blue               | con               |
| December<br>(Laboratory background) | 24 | 0.562 $\pm$ 0.293  | 0.394 $\pm$ 0.295 |
| August<br>(‘Natural’ background)    | 17 | 0.449 $\pm$ 0.274  | 0.496 $\pm$ 0.262 |

**Table 3.** Mean times of association for female test fish with groups containing a rare male phenotype. Times are given as proportions,  $\pm$  SD. See text for details. Times of association of test fish with groups with a rare male vs. groups with all males of the same phenotype were tested against the null hypothesis of equal means by two-tailed paired Student's *t*-test. In neither case did time of association differ from expected under the null hypothesis (rare blue,  $t = 1.219$ ,  $df = 25$ ,  $p > 0.23$ ; rare con,  $t = 0.473$ ,  $df = 25$ ,  $p > 0.64$ ). Differences in time of association of test fish between groups with rare blue males and rare con males were tested against the null hypothesis of equal means with an unpaired *t*-test. Time of association did not differ from expected under the null hypothesis ( $t = 1.558$ ,  $df = 50$ ,  $p > 0.12$ ).

| Rare phenotype | n  | Phenotype of group |                   |
|----------------|----|--------------------|-------------------|
|                |    | rare male          | all same phen.    |
| Rare blue      | 26 | 0.531 $\pm$ 0.253  | 0.416 $\pm$ 0.236 |
| Rare con       | 26 | 0.434 $\pm$ 0.193  | 0.470 $\pm$ 0.204 |

relative absence of algae and 'gelbstoffe' (organic matter resulting from the decomposition of vegetation) sufficiently affected the absorption spectra of the water in the test tank so that the fishes' vision was affected. Ideally, in such experiments the water in the testing environment should match the absorptive qualities of the species natural habitat.

In one experiment, there was a significant side bias, and in two others there was a trend toward a side bias, always to the same side of the tank. Each was in an experiment with the laboratory background. This result supports Endler's (1978) admonition as to the importance of background color pattern in studies of selection on animal color patterns. It is probably not coincidental that our experiments that resulted in the most nearly equal mean times of association were those with the 'natural' background.

In conclusion, we found no evidence for any behavioral differences between phenotypes. Nor did we find any effect of male phenotype on the behavior of test fish, both male and female, when presented with groups of males of a particular phenotype (s). In light of these results, we see several areas of research involving the *X. pygmaeus* color polymorphism that should be pursued. First, careful studies should attempt to determine the effects of predators on male *X. pygmaeus* [preliminary experiments indicate that *X. pygmaeus* cichlid predators do not tend to 'associate' with males of a particular pheno-

type (L. Dries unpublished data)]. Second, females should be presented with pairs of individual males of each phenotype, in an environment carefully controlled for background and water absorption spectra, as noted above. Third, individual females should be tested repeatedly for possible preferences. Female test fish typically displayed a characteristic behavior that differed from that of male test fish, and it is likely that such behavior is related to mating (Ryan & Causey 1989). It is possible that female preference is polymorphic in the population. Finally, the effects of abiotic factors on the different male phenotypes should be investigated. Various studies have found important physiological effects of color pattern in ectotherms, both direct (e.g. Jones et al. 1977) and correlated (Borowsky 1990, Hairston et al. 1992).

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