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Negative Assortative Mating Based on Body Coloration in the Freshwater Platyfish (Poecillidae: *Xiphophorus maculatus*)

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Abstract

The ability of individuals within a population to survive and thrive is highly dependent upon the maintenance of genetic variation and phenotypic diversity, thereby ensuring adaptation to dynamic environments. A fundamental method of maintaining such variation is through a negative assortative mating strategy, in which individuals would be expected to reproductively select members of the opposite sex that exhibit dissimilar phenotypes. Employing three uniform body color morphs, red, yellow and blue, of the platyfish (*Xiphophorus maculatus*), this study was designed to investigate whether *X. maculatus* females would preferentially be attracted to males exhibiting an alternative color, thereby enabling an examination of the effect of male body coloration on mate choice by adult females. Mate choice was determined based on the initial preference of each female, as well as the amount of time females spent associating with each male. Initial preferences were analyzed using a binomial distribution test, and overall preference data using Wilcoxon signed rank tests. Red females initially selected for dissimilar colored males, and spent a significantly larger amount of time associating with blue and yellow males, as did yellow females with red and blue males. Blue females initially selected and spent a significantly larger amount of time associating with red males but, interestingly, showed no selective preference between blue and yellow males. In these experimental trials, the overall strong mate selection exhibited by female platyfish for males of dissimilar coloration is suggestive of a negative assortative mating strategy and provides evidence for the maintenance of color polymorphism in nature populations.

Keywords: *Xiphophorus maculatus*, platyfish, negative assortative mating, courting behavior, mate choice.

Introduction

Population heterogeneity and phenotypic diversity are major factors enhancing the long-term survival of a species and are closely linked to the ability of that species to respond to environmental change (Bazin et al., 2006). One of the evolutionary mechanisms by which populations can maintain such genetic heterogeneity is through mate choice via assortative mating strategies (Pryke and Griffith, 2007). Individuals exhibiting negative assortative mating (i.e. disassortative mating) are expected to select mates that exhibit dissimilar phenotypes (Workman, 1964; Jiang et al., 2013). This selection process not only prevents the loss of newly developed genotypes due to genetic drift, but also encourages the re-establishment of genotypes that have decreased in frequency due to brief, sudden selective pressures (rapid environmental shifts, predation, etc.), potentially preventing the development of a homogeneous population. Conversely, a positive assortative mating strategy would cause individuals to choose mates exhibiting phenotypes similar to their own. This strategy could prevent the incorporation of potentially deleterious alleles into populations during times when environmental conditions are stable (Whitlock and Agrawal, 2009; Agrawal and Whitlock, 2012; Arbuthnott and Rundle, 2012).

Diversity in coloration has been shown to affect species recognition and mate selection in poeciliid fishes (Endler, 1983). Indeed, much of the research examining sexual selection in fish has utilized members of the Poeciliidae, including guppies (*Poecilia reticulata*), swordtails (*Xiphophorus helleri*), and platyfish (*Xiphophorus maculatus*), all of which exhibit a wide range of color variation due to both artificial and natural selection (Basolo, 2006; Porter and Frankel, 2014). Such variation in coloration patterns of certain Central American poeciliids have been shown to have effects on predation and species recognition (Endler, 1983). This also supports the theory of coloration aiding in sexual selection preferences in P. reticulata, specifically a fe-

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male's natural affinity for orange and other coloration patterns (Houde, 1997). Additional research using pygmy swordtails (*Xiphophorus pygmaeus)* has shown that females exhibit preference for males exhibiting a blue coloration and an aversion to yellow males when given a choice between the two (Kingston, 2003). As a member of the Poeciliidae, platyfish are ideally suited for studies involving mate choice due to several factors, including (1) their relatively uniform body morphology and size among males and females (Kallman, 1975; Basolo, 2006); (2) the use of an ovoviviparous reproductive strategy which allows for easy sexing of males and females through the presence or absence of a gonopodium (Rosenthal and De Leon, 2006); and (3) the historically well-documented and easily identifiable courtship and mating behaviors that are exhibited by both sexes (Noble, 1938; Schlosberg et al., 1949; Rosen and Tucker, 1961). Wild platyfish populations are highly polymorphic for color patterns (Borowsky and Kallman, 1976; Basolo, 2006) and, as a result, it is often difficult to isolate mate preferences due to coloration alone using wild individuals. As the popularity of X. *maculatus* in the aquarium trade has grown, new color variants have been developed through artificial selection. While more vivid than those typically found in these populations, careful selection of individuals that display similar color patterns, coupled with the retention of natural mating behaviors, makes them ideally suited for studies involving coloration and mate choice. This study was designed to investigate whether assortative mating for alternative body coloration is observed amongst female X. *maculatus*. It is hypothesized that females will both preferentially select males of alternate coloration and spend significantly more time associating with those males as compared to males exhibiting the female's coloration.

Materials and Methods

To investigate female preference for males of dissimilar body coloration based on three distinct color morphs, a series of experimental trials was conducted employing similar constructs as described for studies on pygmy swordtails (Kingston, 2003). In the work presented here, three phenotypes of X. *maculatus* were chosen, red, yellow, and blue color morphs. These phenotypes were selected for their uniformity in overall coloration from individual to individual within these color variants.

Healthy, sexually mature (>140 d post-birth) individuals of each color variety were obtained from a local retail distributor (G&G Aquatics, Lorton, Virginia, USA). Because previous research using the congeneric X. variatus has shown that body size and length can impact female selection (MacLaren et al., 2011), male size was standardized in this study based on total length (mm) and weight (g). Males deviating beyond one standard deviation in total body length and/or body mass from the general population were not utilized for this study. Males and females that displayed differences in melanin pigmentation or marking expression were also excluded at this time. Selected males and females were separated by coloration and housed in single sex, 75.7 L stock tanks. Because females have been shown to copy the mate choice of other individuals in studies involving other poeciliids (Dugatkin, 1992), the walls of all stock tanks were covered to limit any visual interactions between individuals of various colorations and sexes. This also served to reduce stress from environmental stimuli. Fish were fed a commercial flake diet twice a day until satiated and maintained under a 14 hr : 10 hr (light : dark) photoperiod at a temperature range of 26 ± 10C. Water quality (ammonia, nitrite, and nitrate) was monitored weekly, and the pH was maintained between 7.3 and 7.5.

Experiments were conducted utilizing a standard (56.7 L) capacity aquarium, with clear Plexiglas® partitions placed equidistant from the center of the tank and sealed with aquarium silicone to create two isolated holding areas for the male subjects. These partitions allowed the female in each experiment to visually interact with males in both compartments, but prevented chemical and physical interactions. The back and side panels of the tank were covered to prevent interference from any external stimuli. A black curtain was hung three feet from the front of the tank to further prevent behavioral modification during the trials as a result of external stimuli (Fig. 1).

For each trial, two males from the labeled tanks were randomly selected from the experimental male populations. To account for any side bias, one male exhibiting the female's color morph was placed randomly into one of the side chambers of the experimental tank (Fig. 1). Another male exhibiting either red, yellow, or blue coloration was placed in the opposite chamber. To prevent an order effect, the order in which the various colorations were introduced to the females was randomized. A single female was then placed into a 10 cm x 10 cm x 25 cm Plexiglas® holding chamber located in the center of the tank. After an acclimation period of 2 minutes (during which the female was allowed to observe but not interact with the two males), the holding chamber was slowly and gently removed, and the female was allowed to associate with both males. Courting behaviors and male associations exhibited by the female were recorded using a web camera (Logitech G920) and iSpy recording software (version 6.6.7.0) for an 8 minute period. Using these methods, 28 females were tested for each color combination. To test for side bias, females were also tested with two males of her own coloration on both sides A and B of the experimental tank.

Mate preference trials were conducted under an approved

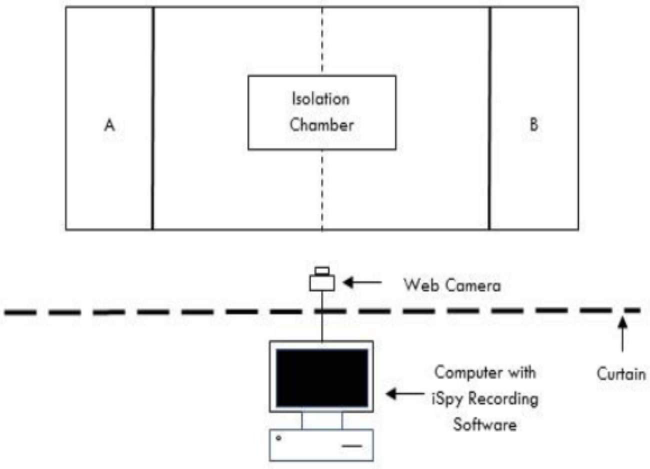

Figure 1. Diagram of behavioral recording setup.

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Howard University IACUC protocol (IACUC-GSAS-11-03). To ensure consistency and prevent observer bias, we utilized a single-blind experimental design. To determine female preference, we recorded the total amount of time (s) each female spent associating with either male, and time was only recorded when both the female and male were actively engaged. We defined association based on the descriptions of platyfish courtship provided by Schlosberg et al. (1949), which include behaviors such as posing, sigmoidal flexing, and arching. The amount of time female *X. maculatus* spend associating with males has been shown to be a reliable indicator of mate selection and final reproductive outcomes in previous studies involving various *Xiphophorus* species (Cummings and Mollaghan, 2006; Walling et al., 2010).

All data analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA). We compared male preference displayed by females of each coloration by subtracting the amount of time spent by each female associating with the alternately colored male, from the amount of time she spent associating with the same colored male, and compared these values to a null expected value of zero using the Wilcoxon signed-rank test (p = 0.05).

Results and Discussion

A total of 252 initial preference and association trials were performed over the course of this study. Females of all color morphs did not show any side bias throughout the course of the study, as indicated by their equal association times when tested against two males of their own coloration.

When tested against red and blue males, red females spent significantly more time associating with blue males than with red males (p = 0.0001) (Fig. 2). Red females also spent significantly more time associating with yellow males compared to red males (p = 0.0028) (Fig. 2). Yellow females spent significantly more time associating with alternately colored blue males (p = .0065) and red males (p = 0.0004) compared to the yellow males used in the trials (Fig. 3). Blue females significantly preferred associating with red males over blue males (p = 0.0032), but showed no significant difference in association time when tested against blue and yellow males (p = 0.7040) (Fig. 4).

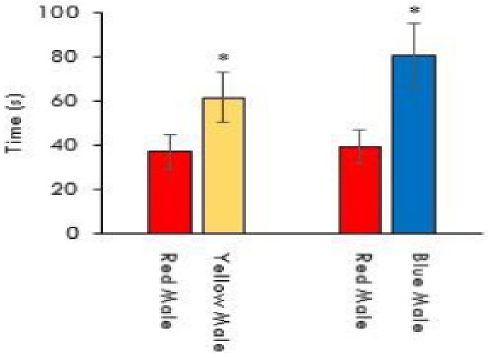

Figure 2. Bars represent average time ± SE red females spent associating with red or yellow males and red or blue males during association trials. Asterisks indicate significant differences (p < 0.05).

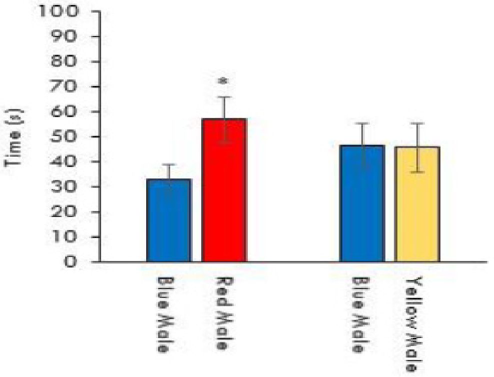

Figure 3. Bars represent average time ± SE blue females spent associating with blue or red males and blue or yellow males during association trials. Asterisks indicate significant differences (p < 0.05).

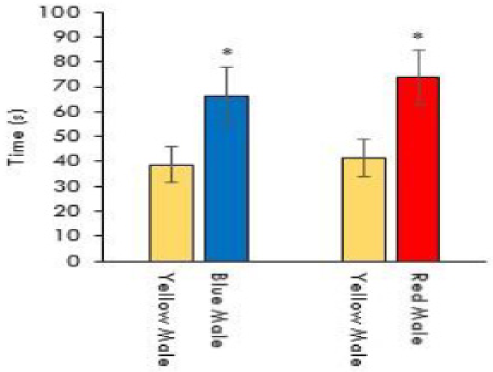

Figure 4. Bars represent average time ± SE yellow females spent associating with yellow or blue males and yellow or red males during association trials. Asterisks indicate significant differences (p < 0.05).

Overall, the results of this study show a distinct association preference of red and yellow female platyfish toward males of dissimilar coloration. Because courtship and display behaviors entail an increased risk of predation in the wild, mate assessment by females must be performed using cues that are readily apparent and virtually instantly assessable (Sullivan, 1994). Based on the results of this study, platyfish appear to generally utilize a negative assortative mating strategy when selecting for males based on body coloration, although the total time each female spent associating with each male varied from individual to individual in all three color morph populations. This, along with the findings of other studies showing varying degrees of affinity for male ornamentation by females (Houde, 1997; Morris et al., 2003), suggests that while a tendency exists for the selection of dissimilar males, there is variation in the existence and strength of female preference. Red and yellow females showed a high affinity towards both alternate color morphs when making an initial selection, and continued to show a significant preference for dissimilarly colored males over time. This finding was repeated when blue females were exposed to red males, but not when blue females were exposed to yellow males. While there have been previous studies examining the effects of morphological characteristics on mate choice in platyfish, this is the first to examine assortative mating in *X. maculatus* based on body coloration. Negative assortative mating has been postulated to be a rare occurrence compared to other mating strategies (Jiang et al., 2013). However, our results provide evidence for the use of a negative assortative mating strategy based on intraspecific advertised body coloration in female platyfish. This result is consistent with the findings of previous studies examining the effects of various dissimilar phenotypes on mate choice in a range of taxa, including coloration in the Siamese fighting fish Betta splendens (Clotfelter et al., 2006), mouth-opening direction in the cichlid Perissodus microlepis (Takahashi and Hori, 2008), plumage coloration in the pigeon Columba livia (Johnston and Johnson, 1989), striping coloration in the white-throated sparrow Zonotrichia albicollis (Houtman and Falls 1994), and body size in the sand lizard Lacerta agilis (Olsson, 1993).

We did not see evidence of a negative assortative mating strategy when blue females were allowed to select between blue and yellow males over time (Fig. 4). This lack of preference in a specific coloration of females for a specific coloration of males is an intriguing finding, albeit difficult to explain. The aversion to gold males has been observed in other studies examining mate choice in the pygmy swordtail (Kingston, 2003) and various neotropical cichlid fish (Elmer et al., 2009). Additionally, researchers examining mate selection in poeciliids have noted an increased risk of predation associated with the display of conspicuous male ornamentation (Endler, 1987; Rosenthal et al., 2001; Godin and McDonough, 2003), and a larger cost to immune system function associated with increased carotenoid pigment expression in gold males (Hill, 1999). Thus, it is possible that the lack of preference by blue females when asked to choose between blue and yellow males is due to the higher risk of predation associated with yellow coloration, or due to the negative impacts of yellow coloration on immune function. The fact that red females showed a preference for yellow males indicates the relative strength of assortative mating, and a possible instance in which the observed female polymorphic preference may have played an active role in altering overall preference for the population.

We recognize that the phenotypes utilized in this study are the result of artificial selection, and would be rare, or likely not found, in natural populations. However, the clear association in our results between alternative coloration and female mate preference illustrates how the relatively subtle, but highly varied, coloration polymorphism (Basolo, 2006) may be maintained in feral populations of platyfish. Further research is required to determine if these preferences for coloration exist within wild populations of this species.

While multiple theories exist regarding the evolution of female choice for alternative phenotypes, the exact mechanisms for the evolution of this preference provides impetus for future research. Most explanations for why negative assortative mating and mate choice exists focus on prevention of inbreeding and maintaining genetic diversity. In poeciliids, traits such as orange coloration (Hughes et al., 2013), fin spotting (Culumber and Rosenthal, 2013), sword length (Basolo, 1990), and body size (MacLaren and Fontaine, 2011) are known to influence female choice and maintain phenotypic diversity in wild populations. That said, our results are inconsistent with previous findings in guppies, in which females were attracted to increasing degrees of one coloration in males (Houde, 1997; Grether et al., 2001; Rodd et al., 2002), suggesting that patterns of sexual selection may be species specific even among members of the same family of fishes.

We contend that the platyfish is a powerful model species for studying mating preferences based on coloration due to their homogeneous body morphology (mass, body length, etc.), readily observable and well documented courting behaviors, and the existing body of knowledge concerning genetic linkages and color polymorphisms (Basolo, 2006; Culumber, 2014). Most importantly, the recent sequencing of the platyfish genome has opened the door for the discovery of genetic underpinnings for the evolution of life history traits such as reproductive behaviors and mate selection (Schartl et al., 2013).

We conclude that the female platyfish utilized in this study follow a negative assortative mating strategy when selecting for male mates based on body coloration. These findings are significant due to their implications involving the maintenance of genotypic variation and should be tested in wild platyfish with different color phenotypes. While this study has examined female preference based on individual male coloration, the presence or absence of a positive or negative frequency dependent selection strategy (where females will select for specific male phenotypes based on their frequency within a population) has yet to be examined and is an interesting subject for future research.

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