Female mating preferences as a result of coloration and movement in *Betta splendens*

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Introduction

It has been found in many species, including Betta splendens (Siamese fighting fish) and Hirundo rustica (barn swallows), that red pigmentation indicates good immunity and health in an individual, which is indicative of their genes and reproductive success (Saino et al, 2000). In addition to this, previous research has shown that female bettas tend to prefer dark red-pigmented males over light red-pigmented males (Clotfelter et al., 2007), as well as preferring red males over blue males (Clotfelter et al. 2007). In some species of fish, such as Poecilia reticulata, color may indicate a severe parasite infestation and thus, bright flashy colors might indicate immunocompetence (Houde et al. 1992). However, severe parasite infestations result in lethargy, which might make a male less willing to expend the energy required to court a female.

Betta fish are well known for their flashy colors and extremely aggressive behavior (Doutrelant & McGregor, 2000). They are an excellent model species for studies of both intrasexual and intersexual selection. If placed in the same enclosure, male bettas will fight, especially if they are protecting a territory containing a bubble nest meant for a receptive female (Noble, 1938). This is an example of intrasexual selection as males are competing against other males for territory, females, defense, and so on. It has also been found that female bettas will "eavesdrop" on fighting males to select the winner, which is an example of intersexual selection (Doutrelant & McGregor, 2000). If receptive, the male will attack a female to determine her readiness to breed (Noble, 1938), thus again indicating intersexual selection. The male must test her readiness to breed, thus determining if she is receptive, fit, and worth the energy it takes to produce sperm and copulate.

Our preliminary observations, in addition to previous research, have shown that females will select males for a number of reasons if there is not an opportunity to eavesdrop, including male flashiness and color, or aggressiveness and movement (Dzieweczynski et al., 2005). What is not known is which of these two categories matters most to the female: color or movement? We hypothesized that female bettas will prefer red males over blue males due to previous research suggesting that red coloration is an indication of health and immunity. We also hypothesize that female betta will prefer movement over color. Thus, it is predicted that female bettas will spend on average more time pursuing blue male betta cutouts that move verses red male betta cut outs that are stationary.

Methods and Materials:

For experiment one, four red males of varying pigment, four blue males of varying pigment, and eight females of various color were obtained to test the hypothesis that female betta preferred red males. The fish's ages were not known and it was unknown if the females were virgins or not. The fish were kept in individual, empty one liter plastic tanks that were filled with filtered and treated water. Paper was placed around each tank to prevent the fish from seeing each other. The bettas were fed an Aqueon Color Enhancing Betta Food once daily and kept on a 12:12 light/dark schedule. Tests were conducted at various hours between 9:00 am and 9:00 pm. Each trial involved a female and two males (one blue and one red). The female's tank was placed between the two males (with paper obscuring her view of the males) and a line of tape was placed to create three zones: a red male zone, a blue male zone, and a center female zone. Three timers were obtained; one timer was used to count down trial time (two minutes per trial), another was used to keep track of the time when the female was observed actively fixated on the red male, and another was used to keep track of the time when the female was observed actively fixated on the blue male. The female was determined to be actively fixated on a male if she swims against the glass separating the two fish with her fins flared. At the start of each trial the papers separating the tanks were removed and the timer was started.

For experiment two, six additional females were obtained to test the hypothesis that female betta prefer movement over color. This brought the sample size to eleven as several females from experiment one were omitted due to not responding. Two paper cutouts were made; one cutout resembled a dark red male betta and another resembled a dark blue male betta. The trials were set up exactly like the first experiment with the exception that males were not present in the tanks. Instead, their tanks were emptied and the cutouts were positioned in the male's place. Paper was still placed between each tank and removed exactly like experiment one to ensure the validity of the experiment. Trials for four different types of conditions were carried out: In the first condition neither cutout was moved, in the second the red cutout was moved mimicking actively fixated male movement, in the third the blue cutout was moved, and on the fourth both cutouts were moved. Female interest was recorded the same way as the first experiment. Trials for the second experiment also ran for two minutes. The female betta were tested in random order and the trial condition was randomly selected. Females were given at least an hour of rest before being tested again. Four females were omitted from data processing due to unresponsiveness to stimuli thus bringing the total sample size to seven.

Results:

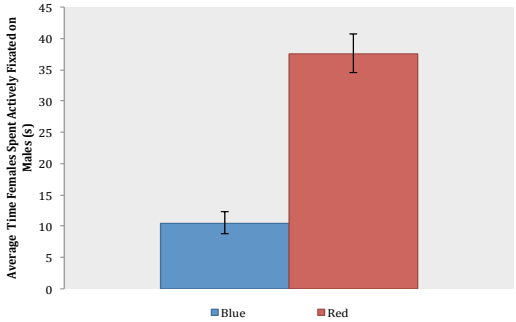

Figure 1. The average amount of time (s) female beta fish spent actively fixated on males of either colour. Error bars represent standard error.

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In experiment one, females spent significantly more time actively fixated on the red males versus the blue males (Fig 1) (t=8.015, df=89, P<0.0001).

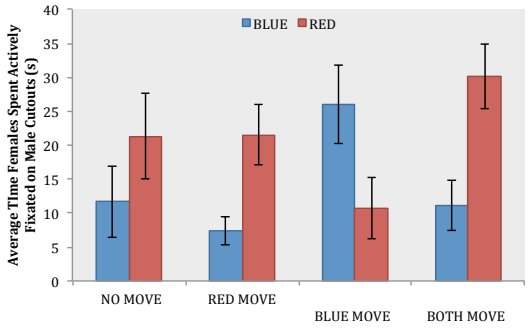

Figure 2. The average amount of time (s) female beta fish spent actively fixated on male cutouts of either colour in four different movement conditions. Error bars represent standard error.

In experiment two, when females were presented with two moving cutouts that resembled a red male and a blue male, they spent significantly more time actively fixated on the red cutout versus the blue cutout (Fig 2) (t=2.526, df=6, P<0.045). When presented with a moving red cutout and a still blue cutout, figure two illustrates how the females spent on average more time actively fixated on the red cutout (t=2.738, df=6, P<0.034). When presented with a moving blue cutout and a still red cutout however, females, on average, spent more time actively fixated on the red cutout. However, this difference was not found to be significant (t=-1.749, df=6, P<0.131). When presented with both non-moving cutouts, the females, on average, did not spend a significant amount of time more actively fixated on either colored cutout (t=1.070, df=6, P<0.326).

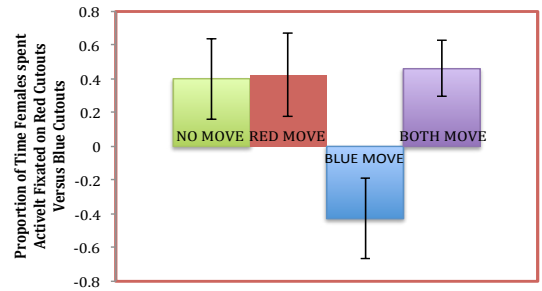

Figure 3. The proportion of time female betta fish spent actively fixated on red cutouts over blue cutouts. Error bars represent standard error.

In order to compare female preference across the fouconditions, we took the time the female spent actively fixated on the red, subtracted by the time actively fixated on the blue, and divided by the total active fixation time to illustrate where the females' preference was directed towards in each condition in the form of a proportion of preference. The proportion of time females spent actively fixated on red cutouts over blue cutouts only differs significantly when the blue cutout was the only cutout in movement (Fig 3) (F=4.233, df=3, P<0.015). A Tukey Post-Hoc test comparing the mean of each condition to the mean of every other condition showed statistically significant differences in means only between the "blue move" condition and the other three conditions (Table 1) (p<0.05).

Dependent \ Data						
Tukey HSD						
					95% Confidence Interval	
(I) Count		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NO MOV	RED MOVE	-.02285	.29515	1.000	-.8371	.7914
	BLUE MOVE	.83001*	.29515	.045	.0158	1.6442
	BOTH MOVE	-.05933	.29515	.997	-.8735	.7549
RED MOVE	NO MOV	.02285	.29515	1.000	-.7914	.8371
	BLUE MOVE	.85286*	.29515	.038	.0387	1.6671
	BOTH MOVE	-.03648	.29515	.999	-.8507	.7777
BLUE MOVE	NO MOV	-.83001*	.29515	.045	-1.6442	-.0158
	RED MOVE	-.85286*	.29515	.038	-1.6671	-.0387
	BOTH MOVE	-.88934*	.29515	.029	-1.7035	-.0751
BOTH MOVE	NO MOV	.05933	.29515	.997	-.7549	.8735
	RED MOVE	.03648	.29515	.999	-.7777	.8507
	BLUE MOVE	.88934*	.29515	.029	.0751	1.7035

*. The mean difference is significant at the 0.05 level.

Table 1. A Tukey post-hoc test comparing the mean of each condition to the mean of every other condition. Green highlight indicates significantly different means.

Discussion

As predicted, when female B. splendens were presented with live males they preferred males with red pigmentation over males with blue pigmentation. This result supports a previous study conducted by Clotfelter et al (2007) in which they also observed that female bettas preferred red colored males over their blue counterparts. Clotfelter et al also found a positive correlation between red pigmentation and immunocompetence in male bettas. These results suggest that females prefer red-pigmented males because they are of better health and have stronger immune systems. A similar pattern has been observed in female guppies (Poecilia reticulata). It was found that female guppies have a stronger preference for males with higher system levels of carotenoid pigments (Houde et al., 1992). Due to the fact that carotenoid pigments are obtained through foraging, high levels of carotenoid pigments may indicate that a male betta is a strong forager (Grether 2000). Knowing this, we propose that female betta prefer red pigmented males because it is an indicator a male's ability to forage, his immune system and associated genes, and overall health. However, it is important to note that all bettas used in our study were obtained from commercial suppliers and their mate preferences may not be indicative of those in wild betta fish. Commercial suppliers breed betta fish with patterns and colorations that consumers are interested in buying and not necessarily those that are common in the wild (Monvises et al, 2009). Future studies might look at whether wild female bettas prefer red males over blue males.

In our second experiment we found that female bettas presented with male cutouts spent, on average, more time actively fixated on the red cutouts than on blue cutouts with the exception of the condition in which the blue cutout was moving and the red was immobile. These results support our hypothesis, which states that when considering a mate, female bettas prefer red pigmentation, but will choose movement over color. We speculate that female betta prefer red cutouts the majority of the time because, as demonstrated in various studies, red pigmentation might indicate good health, superior foraging ability, and immunocompetence (Clotfelter et al 2007; Grether 2000; Putnam 1992). With respect to the third condition; while the females seemed to prefer the blue moving cutout over the unmoving red cutout, this difference was not found to be significant. If the sample size were larger than seven, it is possible that the difference observed between the average amount of time the females spent fixated on either cutout could be significant. The results from the Tukey Post-Hoc test clearly show how the average amount of time the female bettas spent actively fixated on the moving blue cutout is significantly different from all the other conditions' average proportion of time. Thus,

when the blue cutout is moving, the female demonstrates a significantly different preference than any of the other conditions. These results thus show how when considering a mate, female bettas prefer red pigmentation, but will choose movement over color. According to these results, it could be that movement is a crucial part of the betta mating ritual. A stationary male is deemed to be uninterested and, after unsuccessful courtship attempts, the female switches to the moving blue cutout. With this in mind, lethargy in males could be an indicator of poor foraging or immunoincompetent genes, which is undesirable in a mate. This behavior has been previously documented, as guppies infected with a parasite were less likely to partake in courtship behaviors in addition to being more likely to spend time foraging (Kolluru et al 2008). Future studies might conduct trials where a mobile blue cutout is tested against an immobile blue cutout. This, in addition to having a larger sample of females would further solidify the findings found in this study.

This study was partially successful in identifying movement as an important factor in mate choice in B. splendens. Future studies should focus on identifying other factors that influence mate choice in species that also exhibit intersexual selection. Gaining a better understanding of mate preference B. splendens mating may ultimately allow us to better understand mate preference in other species that select their male mates over color, movement, and other visual cues.

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