Variation in male mate preference for female size in
the swordtail *Xiphophorus malinche*

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Summary

Male and female mate preferences have the potential to influence one another, and such interactions could help explain variation in female mate preferences. In *Xiphophorus malinche*, larger females prefer asymmetrical males while smaller females prefer symmetrical males. We used a two-part preference test to determine if there were differences in mate preferences between symmetrical and asymmetrical males for female size that could influence female mate preference. We found no significant difference between symmetrical and asymmetrical male’s preferences. A preference for large female size was detected during the time males directly interacted with females and in standard dichotomous choice tests that followed, as long as the males had been isolated less than 30 days. We did detect variation in male preference for female size depending on male size and the amount of time a male was isolated. These results suggest that variation in male mate preference is not likely to have produced the difference in female preference for symmetry between large and small females, but should be considered where females vary in their preference for male size. In addition, our results suggest that males may shift their preferences from large to small females depending on time since last mating opportunity.

Keywords: male mate preference, symmetry, female size, variation in mate preference, *Xiphophorus*.

Introduction

Not all females prefer the same male or have the same strength of preference. Such variation in female mate preferences has been detected in a wide
range of taxa (Godin & Dugatkin, 1995; Wagner et al., 1995; Gerhardt et al., 2000; Brooks & Endler, 2001; Morris et al., 2003; Rios-Cardenas et al., 2007; Lehtonen & Lindström, 2008). Female experience in multiple contexts can produce variation in preference both across and within females if females do not always respond to the same experience in the same way, or if the experience is different for different females. Studies have shown that male behaviors can produce the type of experiences that alter female mate preferences (male display rates: Marler et al., 1997; male aggression: Patricelli et al., 2004; Ophir & Galef, 2004; subadult experience: Hebets, 2003). For example, given simultaneous experience with two heterospecifics, Amazon mollies exhibit a switch in preference to the species with a higher courtship rate (Marler et al., 1997), illustrating that male courtship rates can influence female preference for male phenotypes.

Although females are thought of as the choosier sex (Darwin, 1871), this does not imply that males lack mate preferences. Males in a wide variety of taxa exhibit mate preferences for female traits, including female ornament coloration (Hill, 1993; Amundsen et al., 1997; Amundsen & Forsgren, 2001; Torres & Velando, 2005), female crest ornaments (Jones & Hunter, 1999), female pheromones (Smith, 1983; LeMaster & Mason, 2002; Carazo et al., 2004) and female size (for review see Andersson, 1994). Of the male mate preferences that have been studied, male preference for female size is the most ubiquitous (Berven, 1981; Cote & Hunte, 1989; Verrell, 1989; Erlanson & Johannesson, 1992; Olsson, 1993; Kraak & Bakker, 1998; Beeching & Hopp, 1999). Studies of livebearing fishes (family: Poeciliidae) have detected male mate preference for larger females (Houde, 1997; Ptacek & Travis, 1997; Basolo, 2004; Herdman et al., 2004; Dosen & Montgomery, 2004), and these preferences were attributed to the increased fecundity of larger females (Abrahams, 1993; Reznick et al., 1993; Herdman et al., 2004). If not all males prefer the same females, this variation in male mate preference would give different females different mating experiences, and could produce differences across females in their mate preferences for male phenotypes. Variation in male mate preference for female traits has been demonstrated to be correlated with male phenotype such that males exhibit positive assortative mate preferences based on color patterns (Roulin, 1999). Variation in male mate preference for female size has been shown in a poeciliid fish (Basolo, 2004) which could provide females of different sizes different
experiences if males of a particular phenotype spend more time associating with or direct more mating behaviors towards larger females.

In a previous study of *Xiphophorus malinche*, a correlation between preference for vertical bar number symmetry and size was detected, in which small females preferred symmetrical males while large females preferred asymmetrical males (Morris et al., 2006). Females in this species continue to grow after sexual maturity (Kallman, 1989; Marcus & McCune, 1999; Morris et al., 2006) and, therefore, the correlation between female size and preference suggests females may change their mating preferences over their lifetime. In the current study, we investigated the possibility that male mate preferences for female body size are correlated with differences in vertical bar symmetry and, therefore, could explain the difference between large and small females in their mate preference for bar number symmetry. If females are more likely to prefer males that prefer them, then we predicted that symmetrical males would be more likely to prefer small females while asymmetrical males would prefer larger females.

**Methods**

Male and female *X. malinche* were collected from the Río Claro in the state of Hidalgo, Mexico in 2005 and 2007. All individuals were fed daily and maintained on a 12 h light/12 h dark photoperiod at a constant room temperature of 22°C. All individuals were measured for standard length (SL), which is the distance from the tip of the snout to the posterior end of the caudal peduncle. Males also were scored for the number of vertical bars on each side, where symmetry indicates equal bar number on both the left and right sides and asymmetry indicates a difference of one bar or more between the left and right sides. Prior to testing, males were individually isolated in 18.9-l aquaria, either directly from the field or from communal tanks. Isolation time is the number of days between the day the male was first placed into a 18.9-l tank and the day the male was tested. This isolation time before testing indicates the amount of time since the male’s last opportunity to mate. A total of 20 males were tested; 14 males (7 symmetrical and 7 asymmetrical) were collected in 2007 and 6 males (1 symmetrical and 5 asymmetrical) were collected in 2005.

A total of 9 female pairs were used as stimuli, large females ranged from 43.7 mm to 56.9 mm SL (mean ± SE = 48.8 ± 1.51 mm), and small females
ranged from 33.1 mm to 38.8 mm SL (mean ± SE = 36.1 ± 0.62 mm). The larger females from each pair were significantly larger than the small females (t-test: $t_{16} = 7.752, p < 0.01$). The small female of a pair was on average 25% (SE = 2%) smaller than the large female.

Prior studies of male mate preferences in livebearing fish using visual dichotomous choice tests alone did not detect male mate preference for females based on size (*Poecilia reticulata*: Herdman et al., 2004; *X. cortezi*: Morris, unpublished data), suggesting males may need to come into direct contact with females to assess the essential cues for male mate choice in live-bearing fish. Therefore, we used a two part choice test that first allowed males to directly interact with females (males could assess olfactory, tactile, and visual cues), before confining females to the end compartments of the choice tank and allowing males to choose females without the confounding effects of female–female aggression and female mate choice (males could still assess olfactory and visual cues). The interaction test was always done first, followed by the dichotomous choice test, so that the information males gained by interacting with females could influence their preferences in the dichotomous choice tests.

Both preference tests were conducted in a 208-l aquarium divided into five equal sections. The two end sections were divided by removable Plexiglass sheets with 0.64 cm diameter hole per 38.7 cm$^2$ to allow transmission of visual and olfactory cues, while the inner three sections were visually divided by lines drawn on the outside of the aquarium. Females were placed into the end sections while the male was placed into a clear Plexiglass tube in the center section of the observation aquarium and allowed to acclimate for 10 min. After the acclimation period all individuals were released into the observation tank. Interactions between the females and the male were recorded for 12 min. We recorded the following mating behaviors that the male directed towards the large and small female: approach female, attempted copulations and display to female. Males in this species darken their vertical bars during courtship (Morris et al., 1995) while using a ‘figure-eight’ display (Ryan & Causey, 1989). Use of these behaviors was scored as display to female. We also recorded the association time, which was the time that the male spent within one body length of the female. In addition, we recorded aggressive behaviors exhibited by both the large and the small female (i.e., bites towards other female and display towards other female).
After the interaction period, each female was re-confined to one of the end sections of the aquarium with a Plexiglass divider with holes. The male was placed back into a clear Plexiglass tube in the center section of the tank for a 10-min acclimation period. The male was then released allowing him to swim freely among the three inner sections. The time the male spent in the two sections adjacent to the females was recorded for 10 min. The females were then placed on the opposite ends of the aquarium and the test was repeated starting with the 10-min acclimation period in order to control for any side biases the male might exhibit.

**Statistical analyses**

For the association times during the interaction period and the dichotomous choice test the strength of preference for the large female for each male was calculated by subtracting the time the male spent associating with the small female from the time that the male spent associating with the large female. Thus, positive scores indicate that the male spent more time with the large female and negative scores indicate that the male spent more time with the small female.

We used a Generalized Linear Model (GLM) in SPSS 11 (SPSS, USA) to examine the effects of male size, isolation period and the interaction of these two variables on male preference for female size. One model was used for the interaction test (GLMINT) and another model was used for the choice test (GLMCHO). Both models included symmetry as a fixed factor, and male size and isolation period as covariates. The following interactions were not significant for either model: male size \( \times \) symmetry, isolation period \( \times \) symmetry and male size \( \times \) isolation period. Therefore, we did not include these terms in either model.

To determine if males exhibited a preference for large or small females we compared the total association time that males associated with the large and small females with a paired \( t \)-test as data were normally distributed. In addition, to determine if males directed more mating behaviors towards the large female during the interaction test we compared the number of displays, approaches and attempted copulations directed at the large as compared to the small female with Wilcoxon signed-ranks tests as data were not normally distributed.
Results

Interaction test

Male vertical bar number symmetry did not influence male preference for female size (Table 1). When symmetrical and asymmetrical males were combined, there was a significant preference for the larger female. This was true when using time spent within one body length of a female as a measure of preference (paired t-test: $t_{19} = -5.549$, $p < 0.001$; Figure 1), as well as when using the number of male displays and male approaches (Wilcoxon signed-ranks test: male display: $z = -2.38$, $p < 0.02$; male approaches: $z = -2.18$, $p < 0.03$; Figure 2). However, there was no significant difference in the number of attempted copulations directed towards the larger female as compared to the smaller female (Wilcoxon signed-ranks test: $z = -1.020$, $p = 0.308$; Figure 3). The lack of a significant difference for copulations may be due to the low occurrence of observed copulations (Only 9 of 20 observations had any copulations, compared to male displays 15 of 20 observations and male approaches 18 of 20 observations).

Using association time as our measure of preference, we examined the relationship between male size and strength of preference (time with larger – time with smaller female). There was a significant relationship between male size and strength of preference, with larger males having a stronger preference for the larger female (Table 1; Figure 3).

Behaviors indicating female–female aggression (i.e., bites towards other female and display towards other female) were rare, occurring in only one test out of 21, so no analyses were conducted for these behaviors.

![Figure 1](image-url)

**Figure 1.** Comparison of the time spent with small vs. large females for both symmetrical and asymmetrical males combined. Bars represent mean value (±SE) of the association time. $p < 0.001$. 
Figure 2. Box plots illustrating the comparison of male behaviors directed towards large and small females. Median scores are indicated by central lines, the interquartile range by the boxes, and 10th and 90th percentiles by whiskers. *$p < 0.05$.

Table 1. The effects of symmetry, male size and isolation period on male preference for female size in *Xiphophorus malinche* (Generalized Linear Model with symmetry as a fixed factor and male size and isolation period as covariates, see Methods).

<table>
<thead>
<tr>
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<th>GLMINT</th>
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<td></td>
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The first model corresponds to the interaction test (GLMINT) and the second model corresponds with the choice test (GLMCHO). Significant effects are in bold. $N_{TOTAL} = 20$ ($N_{SYMMETRICAL} = 8$ and $N_{ASYMMETRICAL} = 12$).

Standard dichotomous choice test

As in the interaction test, vertical bar symmetry did not influence male preference for female size (Table 1). In contrast to the interaction test, however, when all the males were combined, we did not detect a significant preference for larger females (mean association time ± SE: small female = 485.2
Figure 3. The relationship between male size and strength of preference (difference in time spent with small and large females) during the interaction test. Negative scores indicate a preference for small females and positive scores indicate a preference for large females.

Figure 4. The relationship between male isolation period and strength of male preference for female size during choice test. Negative scores indicate a preference for small females; positive scores indicate a preference for large females.

± 65.5 s, large female = 470.0 ± 69.9 s; paired t-test: $t_{19} = -0.116$, $p = 0.91$). However, the number of days that males were isolated had a significant effect on male preference (Table 1; Figure 4). We used the regression for the relationship between strength of male preference and isolation...
period to determine the number of days after which a male was more likely to switch preference between tests (where the line crosses zero on the $x$-axis, or 30 days). Males isolated for 30 days or less exhibited a significant preference for large female size (mean association time ± SE: small female = 338.0 ± 57.7 s, large female = 621.3 ± 75.7 s; paired $t$-test: $t_{11} = 2.199, p = 0.05$), which corresponds to the preferences found with the other two indicators (male displays and approaches to female) of male preference measured during the interaction test. Males isolated longer than 30 days had a significant preference for the smaller female in this second test (mean association time ± SE: small female = 706.0 ± 98.3 s, large female = 243.1 ± 85.9 s; paired $t$-test: $t_{7} = −2.661, p < 0.04$). In other words, males isolated longer that 30 days exhibited a switch in preference from the larger female in the interaction period (first test) to the smaller female in the choice test (second test).

**Discussion**

Vertical bar number symmetry did not influence a male’s strength of preference for larger females, suggesting that male mate preference is not likely to be a factor that influences the difference in female mate preference for bar symmetry between small and large females in *X. malinche*. However, males allowed to directly interact with females had a significant preference for larger females when preference was measured during the interaction time, as well as in the standard dichotomous choice test that followed, as long as they had not been isolated more than 30 days. In addition, the strength of male preference for female size was correlated with male size, with larger males having a stronger preference for large females while small males have a weak to no preference based on female size. These results are similar to those reported in other fish species; a significant relationship between male size and strength of preference for female size was detected in *Oncorhynchus nerka* (Foote, 1988), *Gobiusculus flavescens* (Amundsen & Forsgren, 2003) and *Brachyrhaphis rhabdophora* (Basolo, 2004).

Population genetic models have shown that male mate preferences can spread in a population when males prefer female traits that are indicators of fitness, such as high fertility (Servadio & Lande, 2006). Female size and fecundity are positively correlated in several Poeciliid fishes (Kallman & Borkoski, 1978; Thibault & Schultz, 1978; Reznick, 1993). While both larger and smaller males would benefit from mating with larger, more fecund
females (Andersson, 1994), smaller males may not have the fighting ability to easily gain access to larger females (Moretz, 2003). Therefore, smaller males with no preference for large females (or a preference for smaller females) could have an advantage over small males exhibiting a strong preference for large female size, if the latter rarely gained access to larger females due to male–male competition (Amundsen & Forsgren, 2003; Basolo, 2004), but continued to prefer larger females. The relationship between male size and preference for female size could also influence female preference for male size. In the closely related swordtail X. multilineatus a relationship between the strength of female preference for large males and female size has been detected (Rios-Cardenas et al., 2007). The relationship between female preference for male size and female size has not yet been examined in X. malinche.

Allowing males to interact with females may be necessary to elicit male mate preferences in poeciliid fishes, as previous studies with X. cortezii (Morris unpublished) and guppies (Herdman et al., 2004) did not detect a preference for larger females using visual tests alone. It is unlikely that males are not capable of assessing female size using visual cues alone, as females of several species of swordtail fish have been shown to prefer large males based only on visual cues (Ryan et al., 1990; Basolo, 1995; Morris et al., 1996; Rios-Cardenas et al., 2007). Therefore, we suggest that males prefer larger females due to behavioral, olfactory and/or tactile cues that are correlated with large size, rather than body size per se. Further studies of male mate preference that examine variation in female reproductive state and female size are needed to understand why males prefer larger females.

Measuring male mate preference at the same time that males are being allowed to freely interact with two females can only be used to examine male mate preference if females do not exhibit female–female aggression. We did not detect female–female aggression in these tests, suggesting that female–female aggression is not likely to interfere with a female’s ability to gain access to a preferred male in these fish. In those species where female–female aggression does exist, the standard dichotomous choice test we used after an interaction period would still make it possible to examine male mate preference, as long as the time the males have been isolated in the laboratory is limited. Assessing male mate preference while allowing males and females to freely interact also introduces the possibility that the time a male spent near a female may actually reflect female mate preference rather than male
mate preference. The results from the dichotomous choice tests made it clear that males were making the choice to associate more with the larger rather than the smaller females, even if this choice may have been influenced by female mate preference during the proceeding interaction period.

The other factor that influenced male preference for larger females in addition to male size was the time males were isolated. While all males appeared to prefer the larger female in the interaction test, in the dichotomous choice test that followed, males isolated less than 30 days continued to have a significant preference for the larger females, while males isolated for more than 30 days had a significant preference for the smaller females. One possible explanation is that when males are isolated for more than 30 days, switching their preference from larger to smaller females between the two tests indicates an attempt to maximize the number of females they mated with rather than continuing to try to mate with the initially preferred larger female. Our results suggest that not only will it be important to control for the time males are isolated in male mate preference tests, but that the effect of time since last mating on male mate preference warrants further investigation.

In summary, male mate preference is not likely to produce the variation in female mate preference for symmetry detected in *X. malinche*, as both symmetrical and asymmetrical males preferred larger females. However, the relationship between male size and mate preference could play a role in variation in female mate preference for male size. Our results also suggest that in testing for male mate preferences in live-bearing fish, measuring preference while a male is allowed to freely interacting with two females will produce the same results as tests that first allow a male and two females to interact followed by a dichotomous choice test which controls for female–female aggression and the direct influence of female mate preferences. Finally, as detecting male mate preference in swordtails may require that males can assess multiple female cues (i.e., behavioral, olfactory, and/or tactile), determining how and why large and small females might differ in this respect will provide valuable insights into why male preference for female size varies depending on male size.

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References


