The Implications of Variation in Female Preferences for Swordtail Fishes

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Abstract

Female swordtails show mating preferences for particular pigment patterns and large male size, and there is significant intraspecific variation in these preferences. Here we review the implications of this variation in female preference for the maintenance of variation in male traits. We suggest that variation in female preference may be maintaining polymorphisms in vertical bars in *Xiphophorus cortezi*, fluctuating asymmetries in vertical bar number in both *X. cortezi* and *X. malinche*, and an alternative mating strategy in *X. multilineatus*. While experience appears to play a role in female preferences for one component of the vertical bars, the mechanisms producing variation in female preference are still under investigation. Regardless of the mechanisms involved, our results suggest that variation in female preference may have important implications for the maintenance of variation of male traits in natural populations.
Sexual selection is an important component in the process of natural selection (Darwin, 1871). Based on the mechanism driving sexual selection, this process has been divided into intrasexual (male-male competition) and intersexual (female mate choice) selection (Andersson, 1994). Here we review examples of how the process of intersexual selection, through variation in female mate choice, may affect the evolution of secondary sexual characters in males in such a way as to maintain variation in the male traits. Specifically, we review how female preference may help maintain a polymorphism in the pigment pattern of vertical bars, vertical bar number asymmetry, and alternative reproductive strategies in swordtail fishes (*Xiphophorus*).

The best-known secondary sexual character among swordtail fishes is the sword, which is known to attract females (Basolo, 1990) as well as deter rival males (Benson and Basolo, 2006). However, other characters are also used for mate choice in these fishes, including pigment patterns like vertical bars and male size. All of the northern swordtail species that have vertical bars can change the expression of the bars, and darken their bars during courtship as well as during male-male interactions. Thus, it is not surprising that this secondary sexual character has been shown to both attract females and deter rival males (Morris et al., 1995). Previous studies have also shown female preferences for large body size in several of the swordtail species, including the green swordtail *X. helleri* (Basolo, 1998), *X. nigrensis* (Ryan et al., 1990), *X. pygmaeus* (Hankison and Morris, 2002; Morris et al., 1996; Ryan and Wagner, 1987), and *X. multifilinatus* (Rios-Cardenas et al., 2007). Similarly, female preference for certain body coloration has been shown in *X. helleri* (Franck et al., 2003) and *X. pygmaeus* (Kingston et al., 2003). Therefore, female preference for male secondary sexual traits is common and well established in these fishes.

Although it is generally assumed that female preferences for particular male traits are consistent among individuals in a population and within individuals under different circumstances, a growing number of recent studies are demonstrating that female preferences vary both across individuals and within an individual across contexts. Some of the factors that affect female preferences are related to ecological conditions such as the abundance of predators. In *X. pygmaeus*, females have an aversion to gold males in a population under low predation risk, which results in a preference for blue males. However, in populations with high predation risk this aversion does not exist, hence no mating preference for either color exists (Kingston et al., 2003). *X. helleri* females have been shown to prefer males with spotted sides when the water is turbid but not when the water is clear (Franck et al. 2001).

In addition to ecological conditions, experience has also been shown to affect female preference; in the guppy *Poecilia reticulata* the exposure of females to males with different levels of orange coloration increases their preference for this trait (Breden et al., 1995; Rosenqvist and Houde, 1997). Even when factors such as experience may be constant between populations (e.g. populations with a high proportion of colorful males and a female preference for them; and populations with a high proportion of dull males and no preference for colorful males), female preferences may change over the reproductive lifetime of the individuals. Further studies with guppies showed that, regardless of their experience, young females prefer males with more orange coloration, but old females do not discriminate between males based on their orange coloration (Kodric-Brown and Nicoletto, 2001).

Together, previous studies suggest that variation in female preference may be an important mechanism that selects and maintains variation in male traits, and this appears particularly important for age-related changes in female preference, because ecological variation between populations is not required in such cases. For the first part of this review, we will focus on female preference for components of the vertical bar pigment pattern, and the distribution of this character in relation to female preference both across and within species. For the second part, we will present similar data for body size/coloration patterns in *X. multifilinatus*. However, in this case we present the results from a study that examined the distribution of these characters across natural populations as well as within populations over time.
**Variation in Female Preference for Vertical Bars**

Six of the nine species of northern swordtails have the micromelanophore pigment pattern “vertical bars”. Using video animations and a technique in which bars can be removed as well applied to males without bars (Hoefler and Morris, 1999), female preference for presence/absence of the bars has been examined across all of the northern swordtails. A phylogenetic analysis suggested that this preference was present in the ancestor to this clade (Morris et al., 2007). In those species without bars (X. nigrensis, X. pygmaeus and X. continens), preference ranges from a preference for “no bars” in X. pygmaeus (likely used to distinguish between X. pygmaeus males and sympatric X. cortezi, Hankison and Morris, 2002) to a retained preference for bars in X. nigrensis (Morris and Ryan, 1996). X. continens has a polymorphism in preference for the bars (some females prefer bars and some prefer no bars, Morris et al., 2005) that also appears to be a retained preference from its closest relative X. montezumae. In addition, no preference for bars was detected in X. birchmanni, a species in which all adult males have bars (Morris et al. 2007). Therefore, female preference for the bars is not correlated with the presence/absence of the bars in three of the nine species.

Within those species of northern swordtails that have this pigment pattern, there is extensive variation in the frequency of males with the bars, the expression of the bars in females, and several other components of the bars including thickness, shape, and degree to which the number of bars differs from side to side (bar number symmetry). Female X. cortezi from the arroyo La Conchita, SLP Mexico have been shown to prefer males with the same number of bars on both sides (Morris and Casey, 1998), more bars, bars that were closer together (Morris et al., 2001) and darker bars (Morris unpublished data). And yet, after testing a large number of females from this population, an overall female preference for the bars could not be detected. Upon further examination of the data, it was noted that there was significant variation across females in their preference for the presence or absence of the bars. Females were then tested twice with the same pair of video animations and variation within and across females in strength of preference for the bars were compared. There was significantly more variation across females than within, with some females spending more time with the barred animation and some with the unbarred animation (Morris et al., 2003). As approximately half of the males have vertical bars in the La Conchita population, it was suggested that variation in female preference for the bars could be helping to maintain the variation in males with vertical bars.

Preference for bar number symmetry was also first detected in X. cortezi from the arroyo La Conchita. Using a technique in which bars were removed by freeze-branding, Morris and Casey (1998) demonstrated that females would switch their preference between two males in a pair matched for size depending on which male had the symmetrical number of bars. While this study controlled for overall bar number between the males in a pair, the fact that the asymmetrical male had one side with a bar number that was less than the male with the symmetrical barring pattern called in to question whether or not females actually preferred symmetry or the largest minimum number of bars on one side. In a follow up study, Morris (1998) demonstrated that given a preference between a symmetrical male and an asymmetrical male with more bars (one side the same as the symmetrical male, one side with more bars), some X. cortezi females demonstrated a stronger preference for more bars while others a stronger preference for symmetry. This study was crucial in arguing that X. cortezi females do assess and prefer symmetry, and that the preference detected was not due to a nonlinear preference function for total bar number (see Merry and Morris, 2001; Shettleworth, 1999). This second study also confirmed that the preference for bar number symmetry was not only independent of a preference for bar number, but that there was significant variation in the strength of female mate preference for these two components of the bars across females (Figure 1).

This initial discovery of variation in strength of preference for symmetry led us to examine preference for symmetry in relation to female size (Morris et al., 2006) and the degree of asymmetry within a population. Rauchenberger et al. (1990) had noted that within the cortezi clade the bars on X. malinche males were more asymmetrical (Figure 2) than on males from the other two species (X. cortezi and X. birchmanni). Therefore, we were interested in determining if X. malinche females preferred asymmetry, which could possibly explain the large number of asymmetrical males in this species. We wanted to compare the results of X. malinche to those of X. cortezi, and so we collected more X. cortezi females from La Conchita as well as X. malinche females from the Rio Claro. As it turned out, the females we collected from La Conchita were much larger than those that had been...
collected previously from this site. We found that females over the size of approximately 38 mm from both species preferred asymmetrical males, while smaller females preferred symmetrical males (Figure 3). In addition, we made a more quantitative study of the degree of asymmetry of the bars in these two species. There was no significant difference in number of asymmetrical males across these two species, but in both species the percentage was quite high (percent of males with an asymmetry score of 2 bars or greater, 60% for *X. cortezi* and 53% for *X. malinche*). We suggested that the fact that some females have a preference for asymmetrical males could help maintain this high degree of asymmetry in these two populations.

Overall, studies that have compared female preference for presence/absence of the bars across species as well as preferences of presence/absence of the bars and bar symmetry within species have led to the following insights. Preference for the bars does not appear to be tightly correlated with the trait across species (two species without bars retained preference for bars once bars were lost, and one species with bars has no female preference for bars), suggesting that the preferences measured do not have a strong genetic basis, and/or that any genetic correlation between preference and trait that arises due to assortative mating is easily lost across speciation events. However, the relationship between female mate preference and male trait within populations appears to be stronger. Evidence of a relationship between male aggressive behavior and bar phenotypes may be the key to this correlation. In a recent study which examined factors that could influence a switch in preference from symmetry to asymmetry over the lifetime of *X. malinche* females, Tudor and Morris (manuscript) suggested that experience plays a role in female preference for bar number symmetry, and that asymmetrical males may be more aggressive than symmetrical males. If male aggressive behavior strongly influences female preferences, as we are hypothesizing, correlations between aggressive behavior and sexually selected traits could help maintain variation in these traits if female responses to aggressive males change over their lifetime (e.g. Ophir and Galef, 2004).

Regardless of the mechanism that produces variation in female mating preferences, this variation has the potential to play an important role in the maintenance of variation in male traits. In the second half of this paper, we present results from...
the first of our tests of the hypothesis that variation in female preferences can maintain variation in male traits in a system where the genetic basis to the differences across males has been determined, and the differences are part of an alternative reproductive strategy.

**Alternative Reproductive Strategies in Xiphophorus multilineatus**

The high-backed pygmy swordtail *X. multilineatus* is a species endemic of the Río Coy and its tributaries (Río Pánuco basin), in the state of San Luis Potosí, northeast Mexico (Rauchenberger et al., 1990). As in most species of *Xiphophorus*, size and age at maturation are determined by a sex-linked gene (*P*): little growth occurs after maturation making male size-classes genetically determined (Kallman, 1989). Four size classes are known in *X. multilineatus*, and these differ in their Y-linked *P* alleles (*s*, *I*, *II*, or *L*) and color genes (Table 1). In this species, mating behaviors are tightly correlated with male size. Only males in the genetically smallest size class (genotype X-*s*/Y-*s*) show sneak-chase behavior (referred hereafter as “sneakers”; Figure 4), while male mating behavior of the three largest size-classes consists exclusively of an elaborate courtship display (referred hereafter as “courters”, Zimmerer & Kallman 1989; Figure 4).

For alternative strategies (*sensu stricto* Gross, 1996) characterized by a genetic polymorphism like the ones in *X. multilineatus*, theory suggests that to be evolutionarily stable, these type of strategies must have equal average fitnesses that will be maintained at equilibrium through negative fre-

**Table 1.**

<table>
<thead>
<tr>
<th>Phenotypic size class</th>
<th>Genotype</th>
<th>Color pattern</th>
<th>Standard length range (mm)</th>
<th>Sword?</th>
<th>Sneak-chase behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small (<em>s</em>)</td>
<td>X-<em>s</em>/Y-<em>s</em></td>
<td>Whole body blue or yellow</td>
<td>18-28</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Intermediate-1 (<em>I</em>)</td>
<td>X-<em>s</em>/Y-<em>I</em></td>
<td>Only margins of caudal fin yellow</td>
<td>22-32</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Intermediate-2 (<em>II</em>)</td>
<td>X-<em>s</em>/Y-<em>II</em></td>
<td>Whole caudal fin yellow</td>
<td>26-39</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Large (<em>L</em>)</td>
<td>X-<em>s</em>/Y-<em>L</em></td>
<td>Only margins of caudal fin yellow</td>
<td>32-47</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

*Allele genotypes, Y-linked color patterns, standard length, presence of sword and of sneak-chase behavior of the four size classes of male *Xiphophorus multilineatus*. Adapted to consider Coy, Oxitipa and Tambaque rivers (modified from Zimmerer and Kallman, 1989)
quency-dependent selection. If within a population there are males that court and males that sneak, at low frequencies the average fitnesses of sneakers would be higher than that of courters. However at high frequencies, the fitness of sneakers would be lower than that of courters. At equilibrium, both strategies would have equal fitnesses (Maynard Smith, 1982).

In the green swordtail fish X. helleri, females prefer males with long swords. However, this preference changes with high levels of perceived predation risk when females no longer prefer long swords (Johnson and Basolo, 2003). From the previous example it becomes obvious that the simple negative frequency-dependent model does not take into account variation of female preference, a factor that may also be important in maintaining alternative reproductive strategies. The predicted frequency of male alternatives in the population increases as a function of female preference for that alternative (Figure 5). It is necessary to consider not only the benefits of a particular mate choice, but the costs as well, because the costs can create the opportunity for alternatives. For instance, if females benefit from mate choice, they should prefer males that do not adopt coercive strategies (as sneaking). However, costs mediated by predation risk may decrease female choosiness (Godin and Briggs, 1996). Furthermore, the proportion of alternative reproductive strategies could be stabilized in the absence of negative frequency dependent-selection on male strategies by disruptive selection caused by female mate choice (e.g. if a proportion of females prefer to mate only with sneakers and the rest only with courters; Austad, 1984). For a complete understanding of the maintenance of male alternatives, female choice as well as the interaction between female choice and male behavior must be considered (Alonzo and Warner, 2000; Henson and Warner, 1997; Sinervo and Zamudio, 2001).

The maintenance of alternative reproductive strategies in a population can be due to negative frequency-dependent selection, but can also result from the effects of environmental heterogeneity (spatially oscillating selection), fluctuating selection (temporally oscillating selection), or many other mechanisms for maintaining genetic variability (e.g. heterozygote superiority; Austad, 1984; Calsbeek et al., 2002; Dominy, 1984). For instance, alternative reproductive strategies would be maintained if the selective values of the alternative strategies go up and down depending on the habitat in which they occur. Dispersal between habitat types would maintain the alternative strategies via spatially oscillating selection (Austad 1984). Likewise, alternative strategies may also be maintained if one strategy is at a selective advantage at a low population density (or small population sizes) and the other at a high population density (or large population sizes), and fluctuations in population density (or size) occur at intervals of every few generations (temporally oscillating selection; Austad 1984; Calsbeek et al. 2002). Therefore, as an additional alternative to frequency-dependent selection, variation in space and time of ecological factors, like predation, and/or female preference for example, could be acting to maintain alternative reproductive strategies.

For female preference to have an important role in the maintenance of alternative mating strategies, there are certain conditions that have to exist. First, female preference should be based on one or more of the components of the different mating strategies. Second, this preference should be variable, either across females or within females depending on context (i.e. ecological, experience, age). Finally, this variation in female preference should actually affect the frequency of the male strategies. Confirming that these conditions are met would support the hypothesis that variation in female preference influences the relative frequency of alternative mating strategies.

**Figure 4.** Courter (above) and sneaker (below) males of X. multilineatus. Photos by K. de Queiroz and M.S. Tudor respectively.
Female Preference for Courters

Zimmerer & Kallman (1989) demonstrated that males from the *X. multilineatus* largest size class (genotype X-s/Y-L) produce more offspring per brood than the smallest males in both controlled lab matings of one male and one female, and competitive matings of one courter, one sneaker and two females. As courters spent more time in association with females, Zimmerer & Kallman (1989) suggested that both male-male competition and female preference for courters provide larger males with the mating advantage. Even though large courting males have higher mating success, smaller males may still have equal fitness due to reaching sexual maturity sooner (Ryan et al., 1992). Furthermore, if the relatively higher reproductive success of courter over sneaker males is the result of females preferring these larger males, then variation in female preference for courter males could produce spatial or temporal variation in sexual selection, which may ultimately influence the maintenance of these alternative mating strategies. Note that the relative mating success of small sneakers does not have to be greater than that of large courters to tip the fitness balance in favour of sneakers, as sneaker males have the advantage of reaching sexual maturity sooner. Recent studies (Rios-Cardenas et al., 2007) have confirmed through controlled preference experiments that females of *X. multilineatus* (collected from three different subpopulations) spend more time associated with courters than with sneaker males (Figure 6). Therefore, female preference indeed contributes to the previously detected mating advantage of courter males in *X. multilineatus* (Zimmerer and Kallman 1989), and any spatial or temporal variation in female preference for courters should also produce variation across space or time in this advantage for courters.

Variation of Female Preference for Courters

Rios-Cardenas et al. (2007) found that despite the overall female preference for courters (see above), this preference was variable. Variation of female preference was analyzed with the time spent with a courter male minus time spent with a sneaker male (defined as strength of preference for courter) in two consecutive tests, providing with an average strength of preference (for test one and two). Further analyses revealed that average strength of preference was positively related to female size, with time spent with courters increasing with female size. In addition, the slopes for the relationship between female size and strength of preference of the three studied subpopulations were not different from each other, suggesting that this pattern of female preference variation is constant across different localities. An important implication from this previous study is that for small females, the preference for courters is weak to non-existent.

Spatial and Temporal Variation of Female Preference

In *X. multilineatus* female size distribution varied across space, with subpopulations where most females are relatively small, compared to subpopulations where most females are relatively large (Rios-Cardenas et al. 2007). Female size distribution also varied across time, as the subpopulation that originally had the smallest females was no different from the subpopulations with the largest females after a couple of years (Rios-Cardenas et al. 2007). Together, the results described in this and the previous section suggest that at a particular time, there are subpopulations (with mostly small females) where there is a weak preference for courters; in these subpopulations sneakers would have an en-
hanced mating probability, making them relatively more successful than in subpopulations with large females. Alternatively, other subpopulations, or the same subpopulations at a different time (with mostly large females), have a strong preference for courters; in these subpopulations courters would have a mating advantage over sneakers. Furthermore, assuming that all other variables remain constant, a direct prediction from the previous findings is that subpopulations with relatively higher frequencies of small females will have relatively higher frequencies of sneaker males, and this should be true when comparisons are being made between subpopulations or within subpopulations at different times.

Frequency of the Alternative Reproductive Strategies

The proportion of sneaker and courtier males also varied across space and time and this variation corresponded to variation of female size (Rios-Cardenas et al. 2007). The subpopulation with the smallest females had the largest proportion of sneaker males, while the subpopulation with the largest females had the largest proportion of courtier males. Furthermore, after a couple of years the female size distribution in subpopulations that originally had small females increased; accordingly, the proportion of courtiers also increased in these subpopulations. The correspondence between female preference for courtiers and their relative proportion could be due to factors other than the selection by female preference.

However, Rios-Cardenas et al. (2007) were able to rule out the differences in time to sexual maturity of the two strategies and natural selection for large body size as primary factors.

So far, the correlation between the spatial and temporal variation in female size and the variation in the frequencies of the different male strategies supports the hypothesis that the former influences the latter. Nevertheless, future experiments should attempt to strengthen the support for a causal relationship between these two variables by ruling out other alternatives in natural subpopulations (i.e., variation in predation) as well as examining this relationship in controlled experimental subpopulations.

Conclusions

Variation in female preference for barred and unbarred males in X. cortezi may contribute to the maintenance of both types of males in this species. In addition, variation in female preference for symmetric and asymmetric males with respect to size in X. malinche and X. cortezi and the high frequency of asymmetric males suggests that this variation in female preference may contribute to the maintenance of both types of males in swordtail populations. Regarding the role of variation of female preference in the maintenance of alternative reproductive strategies in X. multilineatus, it has been confirmed that female preference for large males gives courtiers a mating advantage; but it has also been shown that this preference is weak in small females. A mating advantage is not the only component of fitness; in fact sneakers have the advantage of reaching sexual maturity sooner. This advantage coupled with an enhanced probability to mate with small females suggest that variation in female preference influences selection on the alternative male reproductive strategies and thus supports female preference as a potential mechanism for the maintenance of different male reproductive strategies in natural populations.

In summary, correlational evidence suggests that size and ultimately age related variation in female preference may be an important factor maintaining unbarred males, asymmetrical bar patterns, and alternative reproductive strategies in different swordtail species. One of the mechanisms that could be producing this variation in female preference is a size related response in females to male aggression, as evidence suggests that male aggressive behaviors are correlated with the differences in pigment patterns in addition to male size. Further studies should add experimental support to this evidence; in the mean time, we hope these early studies promote the analyses of female preference variation as an important selective force maintaining variation in natural populations.

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