Notes and Comments

Female Disdain for Swords in a Swordtail Fish

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ABSTRACT: Studies of mate choice evolution tend to focus on how female mating preferences are acquired and how they select for greater elaboration of male traits. By contrast, far less is known about how female preferences might be lost or reversed. In swordtail fish *Xiphophorus*, female preference for the sword ornament is an ancestral trait. *Xiphophorus birchmanni*, however, is one species that has secondarily lost the sword. Using synthetic animation playback of "virtual" males, we found that female *X. birchmanni* preferred a swordless conspecific over a sworded heterospecific. Moreover, when offered the choice between a conspecific without a sword and one with a digitally attached sword, females preferred the former. These results suggest female preferences need not always select for elaboration of male traits, and they provide a plausible explanation for the lack of introgression of a sexual trait in a naturally occurring hybrid zone.

Keywords: mate choice, Poeciliidae, preexisting bias, sexual selection, *Xiphophorus.*

Traditionally, studies of mate choice evolution have tended to focus on how female mating preferences are acquired and how they select for greater elaboration of male traits (Lande 1981; Kirkpatrick 1987; Kirkpatrick and Ryan 1991; Pomiankowski and Iwasa 1998; Kokko et al. 2003). There is no a priori reason, however, why female mating preferences must always favor the exaggeration of male sexual ornaments (Wiens 2001). Recent studies demonstrate that female preferences, once acquired, can be secondarily lost or reduced (Holland and Rice 1998; Rosenthal and Servedio 1999; Pfennig 2000; Rosenthal et al. 2002; Morris

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et al. 2005). When this occurs, females either become indifferent with respect to traits that were once under positive intersexual selection or prefer males that express more modest traits. If selection can cause positive directional female preferences to disappear or become relaxed, conceivably, selection can also cause preferences to be completely reversed (Wiens 2001). Under this scenario, male traits that were once favored by females would now be unattractive and selected against. Although implicit in nearly all theoretical models of preference evolution, such a possibility has been subject to few (if any) empirical tests.

The origin of a female preference is often dependent on preexisting perceptual biases (Kirkpatrick and Ryan 1991; Endler 1992; Ryan 1998; Kokko et al. 2003; Macías García and Ramirez 2005). These biases may reflect ecological constraints (Proctor 1991; Endler 1992) or basic properties of nervous systems (Ryan and Keddy-Hector 1992; Rosenthal and Evans 1998; Ryan 1998). Consequently, one might expect such fundamental biases to be highly constrained and impart immutable directionality to mating preferences (Ryan and Keddy-Hector 1992). To what extent, then, can broad-based preferences arising from preexisting biases be modified, if at all?

A classic example of an open-ended, preexisting female preference for a sexually selected trait is found among fishes in the genus *Xiphophorus*. Members of this group have provided some of the most compelling evidence in support of preexisting receiver bias models of sexual selection (Basolo 1990, 1995). A directional, open-ended bias in favor of the sword ornament, a colorful extension of the caudal fin, is an ancestral trait in *Xiphophorus* (Basolo 1995). Evidence also suggests, however, that female preference for the sword is not fixed. For example, female pygmy swordtails *Xiphophorus nigrensis* are indifferent to swords when exercising mate choice; males with long swords were no more attractive than those with more modest swords (Rosenthal et al. 2002). An ancestral female preference for swords can thus be secondarily lost. A complete reversal of preference, on the other hand, has not, as far as we are aware, been shown in any species.

Xiphophorus birchmanni is a small, sexually dimorphic fish in the monophyletic northern, or Río Pánuco Basin, clade of swordtails (Rauchenberger et al. 1990; Morris et al. 2001) that has secondarily lost the sword (Rauchenberger et al. 1990). A closely related sworded species, *Xiphophorus malinche*, hybridizes freely with *X. birchmanni* where the two species are sympatric ("*X. birchmanni*" males with a small [<1 mm] colorless sword [Rauchenberger et al. 1990] are likely to be hybrids [Rosenthal et al. 2003]). If swords are a preferred trait, one might expect them to be favored in hybrid populations and to introgress into populations with predominantly *X. birchmanni* backgrounds (cf. Parsons et al. 1993). The opposite pattern, in fact, is true: in at least one hybrid zone, the Rio Calnali, swords show reduced expression even in populations characterized by traits typical of *X. malinche*, the sworded species (Rosenthal et al. 2003). This pattern suggests that a possible bias against the sword may explain why the trait has failed to spread across the hybrid zone. In this study, we test the possibility that the ancestral female preference favoring the sword in swordtail fish may, in fact, be reversed in *X. birchmanni*.

Methods

Animal Collection and Housing

Xiphophorus birchmanni were collected from the Rio Garces, Hidalgo, México, a population allopatric with other swordtails (Rauchenberger et al. 1990; G. G. Rosenthal, unpublished data). To ensure that sexually mature females were also sexually receptive, we isolated females from males for at least 3 wk before testing and housed them in well-planted 37.5-L aquaria (Wong et al. 2005). Animals were maintained on a 14L : 10D cycle and fed TetraMin flakes, brine shrimp, bloodworms, and mysids.

Experimental Procedures

Computer animations of "virtual" males were used as stimuli (see below) to test the preferences of live *X. birchmanni* females. Fishes, and swordtails in particular, have proven highly amenable to mate choice studies employing synthetic computer animation playback. By using computer-animated fish, we can control both the appearance and behavior of the stimulus (Künzler and Bakker 1998; Rosenthal et al. 2002), allowing us to directly manipulate the trait(s) we are interested in testing and, more importantly, eliminate variation in other traits or behaviors that could confound female preferences.

We conducted two separate experiments, using a different set of *X. birchmanni* females in each (mean standard length ± SD of females = 35.13 ± 2.88 mm). In experiment 1, females ($n = 18$) were offered the choice between a male *X. birchmanni* stimulus (standard length = 46.3 mm) and a male *Xiphophorus malinche* stimulus (standard length = 46.3 mm, sword length = 7.3 mm). In experiment 2, females ($n = 18$) were offered the choice between two *X. birchmanni* male stimuli (standard length of each male = 46.3 mm), one without a sword and one with a *X. malinche*–type sword (sword length = 7.3 mm).

Computer animations were created using techniques described by Rosenthal (2000*b*) and Rosenthal et al. (2002). Briefly, for experiment 1, *X. birchmanni* and *X. malinche* male stimuli were generated using average trait values for each species on the basis of field measurements of standard length, body depth, sword length, and the width of the dorsal fin (Rosenthal et al. 2003). A representative male from each species was used to generate body texture and vertical bars. *Xiphophorus birchmanni* and *X. malinche* both perform a simple, conserved courtship display in which males raise their dorsal fins and remain in a fixed position in front of the female, quivering slightly. Animations showed males repeatedly swimming onscreen, performing this courtship behavior, and then swimming offscreen. In experiment 2, we used the same *X. birchmanni* male stimulus and digitally attached the *X. malinche* sword to produce the "sworded" stimulus. The sword of *X. malinche* reflects minimally in the ultraviolet (Cummings et al. 2003), so the absence of ultraviolet information in the stimulus was unlikely to affect the response of *X. birchmanni*. Furthermore, previous studies using video playback with *Xiphophorus* (Rosenthal and Evans 1998; Trainor and Basolo 2000) have shown the same preferences for swords on video as are found for swords on live males.

Female preferences were tested in an aquarium measuring (length × width × height) 75 cm × 30 cm × 30 cm. The test aquarium was divided lengthwise into three equal sections (left and right "preference" zones and a central "neutral" zone) by lines drawn on the sides of the tank. Animation sequences were played from a Dell OptiPlex GX260 computer connected to two 35-cm IBM CRT monitors placed at opposite ends of the aquarium, with the screens facing into the tank. Females were individually acclimatized in the test tank for 10 min before the start of each test. After the acclimation period, each female was presented with 10 min of monochromatic screen from both monitors, followed by simultaneous presentation of the 5-min test stimuli. The female was then presented with another 10 min of monochromatic screen followed by 5 min of the same stimulus set, this time switched between monitors to correct for side bias.

We measured the total amount of time each female spent associating with each stimulus in the "preference" zones (i.e., left or right side of aquarium). Association preferences are widely used to estimate mating preferences in

fish (for review, see Forsgren 1992), including swordtails (Basolo 1990, 1995, 1998; Rosenthal and Evans 1998; Hankison and Morris 2002; Rosenthal et al. 2002). In *Xiphophorus*, association preferences are a reliable predictor of mating behavior: when fish were allowed to freely interact, females directed more sexual behavior toward males with whom they had spent more time during association trials (M. E. Cummings and D. Mollaghan, unpublished data). Association preferences in a mating context are also different from those seen in swordtails associating with conspecifics in a shoaling context (Wong and Rosenthal 2005). Females that spent more than 90% of the total time in any one section (left, right, or center) of the aquarium (i.e., sitting passively on the bottom of the aquarium or floating listlessly beneath the water's surface) were defined as unresponsive and were excluded from analysis (Rosenthal et al. 2002).

Results

In experiment 1, females spent significantly more time associating with the *Xiphophorus birchmanni* stimulus compared with the *Xiphophorus malinche* stimulus (Wilcoxon: $z = 3.03$, $n = 18$, $P = .002$; fig. 1*a*). In our second experiment, when offered a choice between the two *X. birchmanni* stimuli, females showed a significant preference for the male without the sword (Wilcoxon: $z = 2.59$, $n = 18$, $P = .01$; fig. 1*b*).

Discussion

Xiphophorus birchmanni females in our study appear to show a complete reversal of the ancestral preference for swords, corresponding to the secondary loss of the sword ornament in males (Rauchenberger et al. 1990; Morris et al. 2001). Not only did females prefer an unsworded conspecific over a sworded *Xiphophorus malinche* stimulus, but when offered a sworded versus an unsworded conspecific stimulus, females actually preferred the latter. This apparent preference reversal contrasts with the secondary reduction of preference for the sword ornament reported in another swordtail, *Xiphophorus nigrensis* (Rosenthal et al. 2002). In that species, males are polymorphic for sword expression, but males with longer swords were no more attractive to females than males with shorter swords. Interestingly, however, female *X. nigrensis* did show a bias against swords when presented with synthetic animations of a male with a supernormal sword characteristic, namely, a digitally attached sword of a congener, *Xiphophorus helleri*. The bias against a *helleri*-type sword could, however, be a product of phylogenetic distance, since *X. nigrensis* and *X. helleri* belong to different clades. Importantly, in this study, the sword presented to female *X. birchmanni*
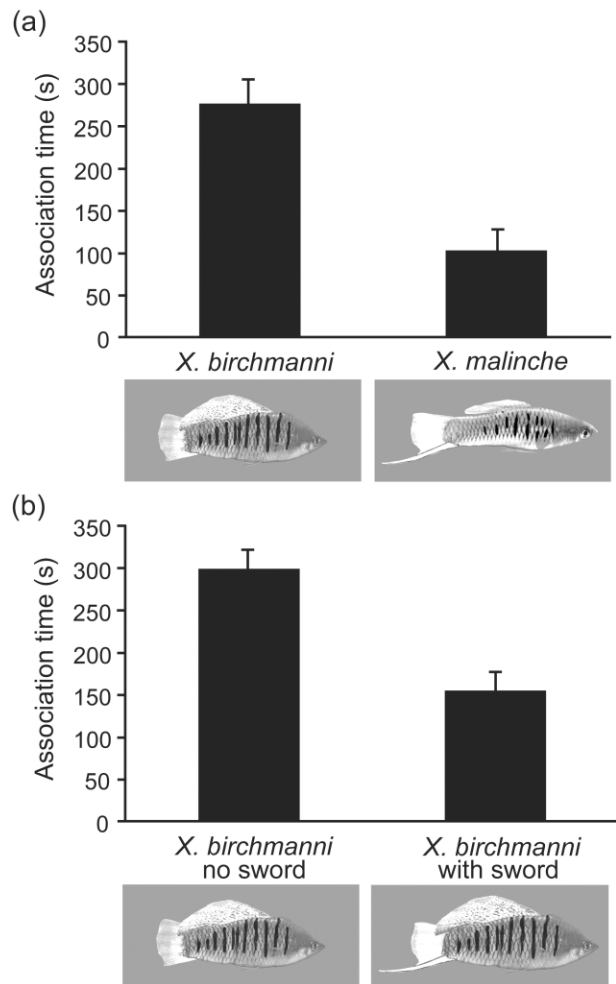

Figure 1: Female association time (mean ± SE) with synthetic animation of (*a*) a courting conspecific versus heterospecific and (*b*) a sworded versus unsworded conspecific.

came from *X. malinche*, a closely related member of the same clade, which, according to several phylogenetic hypotheses, is also its sister species (Rauchenberger et al. 1990; Morris et al. 2001). Thus, the sword offered to females in our study should more closely approximate the ancestral *X. birchmanni* sword and is thus a more realistic ornament for assaying the reversal of female preferences for the trait.

A possible bias against sworded males by *X. birchmanni* females in our study offers some intriguing insights into the distribution of phenotypes across a naturally occurring hybrid zone between *X. birchmanni* and *X. malinche* (Rosenthal et al. 2003). On the basis of other hybrid systems, traits favored by sexual selection are expected to rapidly cross a hybrid zone as reported, for example, in manakins (Parsons et al. 1993). Selection against the sword, however,

may explain the lack of introgression of the sword in the *birchmanni-malinche* hybrid zone reported by Rosenthal et al. (2003). In this regard, it could be interesting to examine whether the strength of female responses in *X. birchmanni* differs between populations depending on whether they are allopatric or sympatric with *X. malinche* (Pfennig 2000).

Female preferences for male traits can often originate from latent perceptual biases that exist outside the context of mating (Kirkpatrick and Ryan 1991; Proctor 1991; Endler 1992; Basolo 1995; Ryan 1998; Rodd et al. 2002; Christy et al. 2003; Macías García and Ramirez 2005). In a review of the literature, Ryan and Keddy-Hector (1992) found that most mating preferences were directional: females showed open-ended preferences for males expressing larger, brighter, faster, or lower-frequency traits. Such open-ended preferences, they argued, might simply arise from the greater stimulation these stimuli provided to perceptual systems. In order to reverse an open-ended preference for a trait, females would have to either lose the underlying perceptual mechanism or, more plausibly, evolve more sophisticated cognitive or perceptual filters allowing them to avoid such stimuli in the specific context of males.

Another possibility that remains to be explored is how female preference for the sword ornament (or lack thereof) might be affected by prior experience. Studies in several animal taxa have shown that female responses to particular traits in males can be affected by prior exposure to those traits as juveniles (ten Cate and Vos 1999; Hebets 2003; Witte and Sawka 2003). Is it possible that the preferences of wild-caught *X. birchmanni* females in our study might simply be due to prior experience with unsworded males (and not because of any secondary reversal of a preexisting female bias for swords)? Future studies could explicitly investigate such a possibility by testing, for example, the response of females raised in the absence/presence of sworded (heterospecific) and unsworded (conspecific) males. It is worth noting, however, that in several species of *Xiphophorus* (as well as members of the sister genus *Priapella*), females show a preference for sworded stimuli even though males in their own populations (with whom they should be most familiar) lack swords (Basolo 1990, 1995). It seems unlikely, therefore, that a female preference for the unsworded stimuli in our study could be due simply (or exclusively) to a preference for familiar-looking (unsworded) males.

Why, then, might preferences be reversed? Although a plethora of studies have considered how preferences for elaborate traits are acquired and maintained, far less attention has been paid to how female preference, once acquired, might be lost or reversed (Wiens 2001). The possible reversal of a preference reported here could be the result of one, or a combination, of causal agents. Reversal of preferences may, for example, occur if choice incurs a high cost (Kokko et al. 2003). For instance, *X. birchmanni* occurs in sympatry with *Astyanax mexicanus*, a predator of swordtails that has a visual preference for males with sword ornaments (Rosenthal et al. 2001). In at least two other swordtail species, populations from high-predation sites show a reduced preference for swords (*X. nigrensis*: Rosenthal et al. 2002; *Xiphophorus pygmaeus*: Rosenthal 2000*a*). Reversal of a preference could also be the result of historical selection events operating against heterospecific matings (Pfennig 2000; Hankison and Morris 2002) as a response to environmental changes affecting signal perception (Seehausen et al. 1997), as a result of genetic drift (Rosenthal et al. 2002), and/or as a product of sexually antagonistic coevolution (Holland and Rice 1998). Regardless, the bias for swords appears to have a relatively recent origin (Basolo 1998; Johnson and Basolo 2003) and, given our results, could potentially be susceptible to loss or reversal. Future studies should address both the mechanisms and the selective agents responsible for the reversal of preferences arising from preexisting perceptual biases.

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