Secondary reduction of preference for the sword ornament in the pygmy swordtail *Xiphophorus nigrensis* (Pisces: Poeciliidae)

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Female mating preferences can be secondarily lost for a number of reasons. We examined the preference of female pygmy swordtails, *Xiphophorus nigrensis*, for the sword, a conspicuous extension of the caudal fin in some males. Females failed to show a preference for conspecific males with swords when presented with live males naturally varying in sword length, with live males of manipulated sword length, and with synthetic animations of males expressing natural variation in sword length. Females showed a significant bias against swords when presented with synthetic animations bearing supernormal sword characteristics. The reduced preference for swords, relative to closely related fish, may result from an increase in the cost of choice due to predation risk, selection against mating with heterospecifics, or changes in the spatial and contrast properties of the conspecific signal.

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Theoretical and empirical work on the evolution of mate choice has emphasized the acquisition and maintenance of preferences for elaborate traits. Yet once acquired, female mating preferences can be lost for a number of reasons. Selection favours the loss of a preference if the preference causes females to mate at a suboptimal rate (Holland & Rice 1998), with heterospecifics (Pfennig 2000), or with phenotypically or genetically inferior males (Zahavi & Zahavi 1997). Loss of preference can occur due to genetic drift, particularly if males in a population do not display variation in the relevant trait. Preferences may also be lost, or unexpressed, if predation or other factors impose a high cost on choice (Forsgren 1992; Berglund 1993, 1994; Hedrick & Dill 1993; Godin & Briggs 1996; Rand et al. 1997) or if signals cease to be perceived appropriately due to changes in the environment (Seehausen et al. 1997). Several studies have shown interspecific variation in the strength of female mating preferences for elaborate traits (McClintock & Uetz 1996; Morris et al. 1996; Basolo 1998a), but in these cases it has not been clear whether weaker preferences represented an ancestral or a derived condition. Identifying evolutionary patterns of preference reduction is critical to an understanding of the evolution of mating preferences.

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Demonstrating a loss of preference is problematic (Rosenthal & Servedio 1999). Unless a preference is altogether reversed with respect to the ancestral state (for example, a novel preference for small over large males rather than vice versa), one must effectively assert the null and draw inferences from negative results. Since differences in preference as low as 5% can cause rapid evolution of male traits (e.g. Servedio & Kirkpatrick 1996), achieving the statistical power necessary to exclude a biologically relevant preference is usually unrealistic. Negative results can, moreover, arise from inappropriately motivated experimental subjects: females overly or insufficiently motivated to mate will often fail to discriminate among potential partners. Deficiencies in experimental stimuli, such as inappropriate lighting conditions or unrealistic artificial models, may also produce spurious negative results. Another, more insidious problem arises from the fact that signals themselves tend to be highly labile. If descendant females fail to prefer a trait in descendant males, it may reflect a change in the male trait rather than evolution of the preference. Moreover, communication signals cannot be divorced from their context: the same signal can be perceived in different ways depending on the signalling environment (McDonald et al. 1995). The perception of a particular trait often depends on other traits: differences in background body colour, for example, can make a patch of pigment more or less conspicuous (Endler 1991).

Therefore, to demonstrate a reduction in preference experimentally three conditions must be met: (1) descendant taxa show preferences that are measurably

weaker than ancestral preferences; (2) female motivation reflects the range found in nature; (3) females appropriately perceive both ancestral and descendant versions of the signal. The present study demonstrates a secondary loss of preference for a conspicuous ornament, the sword, in the poeciliid fish *Xiphophorus nigrensis*.

Background and Experimental System

Males in some species of swordtails (genus *Xiphophorus*) express conspicuous, pigmented extensions of the caudal fin. Female green swordtails, *X. helleri*, prefer males with longer swords (Basolo 1990a; Rosenthal & Evans 1998; Trainor & Basolo 2000). This preference appeared prior to the origin of the sword itself: females of swordless platy-fish (*X. maculatus*, Basolo 1990b; *X. variatus*, Basolo 1995a) and *Priapella olmecae* (Basolo 1995b), which diverged from swordtails prior to the origin of the sword, prefer conspecific males with artificial swords attached. This preference appears to reflect a more general bias: the preference of female *X. helleri* for swords is abolished when the size of a swordless male is augmented to match that of a male with a sword (Rosenthal & Evans 1998). Incrementing sword length carries a far lower metabolic cost than adding body size (Basolo 1998b). Satisfying a female preference for swords thus appears to be an inexpensive means of enhancing male attractiveness (Rosenthal & Evans 1998).

Despite the advantage of expressing an inexpensive ornament that elicits an ancestral preference, swords have been secondarily lost up to three times in the Río Pánuco or northern swordtails alone (Marcus & McCune 1999), and the expression of swords varies considerably among species, populations and individuals. This pattern may be explained in part by variation in predation pressure (Rosenthal 2000a).

Variation in sword expression may also result from preference evolution. Basolo (1998a) argued that preference for swords is weaker in *X. helleri* than in *P. olmecae*, suggesting that preference has been evolutionarily modified subsequent to the origin of the trait. Basolo (1998a) identified two circumstances that might lead to weakening of preferences in *Xiphophorus*. Selection against sword expression, or against longer swords, can cause a reduction or loss of preference if there is a genetic correlation between sword expression or sword length and the female preference. Alternatively, the female bias for swords may be selected against in another context, such as foraging or predator avoidance.

The northern swordtails show considerable geographical variation in female preference for various traits, including body size (Morris et al. 1996), flanking vertical bars (Morris et al. 1995; Morris & Ryan 1996; Morris 1998) and olfactory cues (McLennan & Ryan 1997, 1999). These findings suggest that female preference for swords may also vary across the genus.

The form and colour of the sword vary markedly among swordtail species. The sword is a composite trait made up of four components, each of which can be expressed to varying degrees within a species or individual; briefly, these include an elongation of the lower caudal rays, melanophore coloration on the lower margin of the caudal fin, melanophore coloration on the upper elongated rays, and pterin and/or carotenoid coloration forming a stripe in the centre of the elongated area (Basolo 1995a). In *X. helleri*, the sword is broad and characterized by a conspicuous, green, yellow and/or orange stripe, which contrasts with the adjacent melanophore pigment (Basolo 1990a, 1998a). The artificial sword used to test ancestral preferences in platyfish and *Priapella* resembles that of *X. helleri*, with upper and lower black stripes and yellow internal coloration (Basolo 1990b, 1995a, b, 1998a).

The specific epithet of *X. nigrensis* means 'black sword' (Rauchenberger et al. 1990); its sword is thinner than that of *X. helleri* and shows far less internal contrast to the human eye. Male *X. nigrensis* are polymorphic at a Y-linked locus, the *P* locus, associated with body size and age at maturity. Twenty-five per cent of males lack swords altogether, and the majority (52%) of males have swords less than 10% of standard length. Swords are absent from nearly all males in the smallest (*P-s*) size class, which display sneak/chase copulatory behaviour. Swords are longer and more likely to be expressed in the largest (*P-L*) than in the intermediate (*P-I*) size class (Ryan & Rosenthal 2001). In *X. helleri*, swords range from 70 to 130% of standard length (Basolo 1998a), while in *X. nigrensis* (N=241) the range is 0–57%. Swords in *P-L* males have a mean (±SE) sword index (sword extension length/standard length) of 0.17 ± 0.12 (N=74); for all males, the mean sword index is 0.12 ± 0.11 (N=241). In both length and internal contrast properties, the sword of *X. nigrensis* thus appears to be substantially less conspicuous than that of *X. helleri*.

Reduced sword length in *X. nigrensis* may reflect predation pressure, a reduction in female preference, or both. We evaluated female response to live males varying in sword length and live males with manipulated sword length. We subsequently used playback of synthetic animations to test responses to natural and supernormal variation in sword length and contrast properties, including swords bearing *X. helleri*-like characteristics. Despite the very different methodologies used in these experiments, the results were consistent across experiments; when presented with males with swords within and outside the natural range of variation, females did not prefer longer swords.

EXPERIMENT 1: UNMANIPULATED LIVE MALES

Methods

Mature female *X. nigrensis* were housed together in 40-litre aquaria well provisioned with plants, and separated from males for at least 30 days prior to testing (Morris et al. 1996). Animals were maintained on a 14:10 h light:dark cycle and fed daily TetraMin flake food occasionally supplemented by live brine shrimp and bloodworms. Fish were either wild-caught or recently derived (1–3 generations) from the Nacimiento Río Choy population (21°59′18″ N, 98°53′2″ W), San Luis Potosí, Mexico. Analysis of responses from more than 2000
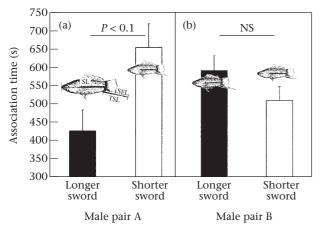
Figure 1. Female association time ($\bar{X}$+SE) with unmanipulated males varying naturally in sword length. (a) Male stimulus pair A; (b) male stimulus pair B. Standard measures of sword length and body size are shown in (a): SL, standard length; TSL, total sword length; SEL, sword extension length. Line drawings not to scale; the longer-sworded male in (a) is enlarged to show measurements.

choice tests in another study yielded no detectable differences in response between wild-caught and laboratory-reared animals (Rosenthal 2000a). We used a new set of females for each experiment reported here. Tests were conducted in 1987.

We selected two pairs of male *X. nigrensis* closely matched for standard length (SL), but varying in total sword length (TSL; Fig. 1a). Total sword length is the distance from the tip of the longest caudal ray to its insertion in the caudal peduncle. Stimulus males in these and subsequent experiments were *P-L* genotype males, with large body size and high rates of courtship display (Ryan & Rosenthal 2001). Male pair A measured 31.4 mm SL and 20.0 mm TSL versus 31.5 mm SL and 12.7 mm TSL, while male pair B measured 30.3 mm SL and 17.1 mm TSL versus 31.2 mm SL and 8.0 mm TSL. Female preference was tested using methods described in Ryan & Wagner (1987). We used an aquarium (45 × 90 cm and filled to a depth of 41 cm) divided into five equal sections along its length. The sections at each end were separated from the three central sections by Plexiglas dividers. We placed a male in each of the end sections. We placed a female in the middle section in an opaque cylinder (11 cm in diameter) and allowed her to acclimatize for 10 min. We then removed the cylinder and recorded the amount of time the female spent in each of the three sections, either the centre section or one of the sections adjacent to a male, for the following 10 min. To control for side biases, we then repeated the trial after switching the males between the end sections of the tank. In this and subsequent experiments, we excluded from analyses females that spent more than 90% of the response time in any one section; less than 10% of females were excluded using this criterion. The end sections of the tank were illuminated by incandescent bulbs. The sides of the aquarium were lined with black plastic. We used a paired *t* test to evaluate the a priori hypothesis that females prefer males with longer swords. We conducted separate tests for each pair of males.

Results

Females failed to prefer the longer-sworded male of either stimulus pair. There was a tendency for females to favour the shorter-sworded male of stimulus pair A (Fig. 1a; paired *t* test: t_{10}=1.89, two-tailed P=0.08) whereas they displayed equivalent responses to males in pair B (Fig. 1b; t_{19}=1.06, N=20, P=0.30). Our failure to detect a preference may have resulted from preference for other traits, such as courtship or body colour, masking a preference for swords, or from testing too small a difference in sword length. We therefore repeated this experiment using pairs of males in which one member of the pair had the sword surgically removed.

EXPERIMENT 2: MANIPULATED LIVE MALES

Methods

Animals were maintained as in experiment 1. Experiments were conducted in 1997. Males were from Nacimiento stock; females came from this population or Estación Hidrometrica (21°59′52″ N, 98°52′20″ W), a site further downstream. We selected as stimuli two pairs of *X. nigrensis* males closely matched for standard length (snout to hypural plate) and original sword extension length (SEL; Fig. 1a). In these and subsequent experiments, we used sword extension length, rather than total sword length, as a measure of sword size. Sword extension length measures the protruding portion of the sword: the distance from the intersection of a line perpendicular to the axis of the ventral-most unextended caudal ray and crossing its tip with the ventral margin of the caudal fin, to the tip of the longest caudal ray. One pair consisted of a male of SL 32.8 mm and SEL 12.6 mm and a male of SL 32.9 mm and SEL 9.9 mm; the other pair consisted of a male of SL 33.3 mm and SEL 10.4 mm and a male of SL 33.6 mm and SEL 9.3 mm. We then anaesthetized males using clove oil suspension and used a sterile scalpel blade to surgically remove the sword extension from the shorter-sworded male in each pair (Basolo 1990a, b, 1995a). We controlled for handling effects by applying the same procedure to the distal end of the other male's sword, removing approximately 0.5 mm of tissue. Swords regenerate rapidly after removal; they are frequently bitten off during encounters with competitors and predators in the field, and 63–86% of adult male *X. nigrensis* in the field show signs of sword damage (T. Flores M., G. G. Rosenthal, F. García de L. & M. J. Ryan, unpublished data). All animals recovered rapidly from anaesthesia. The behaviour of surgically manipulated males in this experiment, and the responses of female test subjects to these males, were qualitatively indistinguishable from those of the unmanipulated males in the first experiment.

To measure female preference, we used a set-up similar to that described for experiment 1, except as follows. The aquarium measured 120 × 32 cm and was filled to a depth of about 40 cm. Two standard fluorescent light bulbs provided illumination. The sides and rear of the aquarium were covered with black felt. The aquarium was
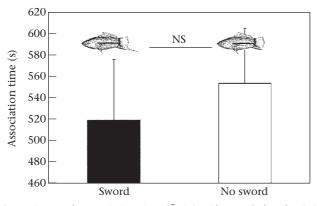
Figure 2. Female association time ($\bar{X}$+SE) with sworded males (■) versus males with swords surgically removed (□).

again divided into five sections; the flanking compartments for the males were 21 cm wide, the 'preference' areas adjacent to the males were 27 cm wide and the central area was 23 cm wide. We used a 12-cm diameter clear Plexiglas cylinder to acclimatize females for 10 min prior to testing. A small clump of Java moss, *Vesicularia dubyana*, was provided for cover in the centre of the aquarium. Unresponsive females typically spent a majority of time in the vegetation. We again recorded female behaviour for 10 min and then repeated the trial with the males switched to control for side biases. We alternated the side of first presentation, such that the sworded male was initially on the left side in every other trial. To minimize habituation of stimulus males, we used each pair in alternate trials. We tested each female with one male pair only. We first tested for differences in net response to each of the pairs with a Mann–Whitney U test, and then pooled the data and used a Wilcoxon matched-pairs signed-rank test to evaluate the a priori hypothesis of sword preference.

Results

There was no detectable difference in response to the two male pairs (Mann–Whitney U test: $U=49$, $N_1=10$, $N_2=11$, $P=0.67$), or between Nacimiento and Estación Hidrometrica populations ($U=36$, $N_1=10$, $N_2=11$, $P=0.74$), thus we pooled data for analysis. Females failed to prefer sworded over unsworded males (Fig. 2; Wilcoxon matched-pairs signed-rank test: $Z=0.50$, $N=21$, two-tailed $P=0.61$), and 12 out of 21 females spent more time with the swordless male.

Females thus failed to show a preference even when presented with the extremes of natural variation in sword length. Although we did not observe any qualitative difference in courtship level between the two males in a pair, or between manipulated and unmanipulated males, a behavioural difference could have resulted from modifications of behaviour as a result of surgery or to compensate for a missing sword. In addition, the background and lighting conditions used in the laboratory may have differed from those under which females perceive swords in nature.

EXPERIMENT 3: NATURALLY VARYING SYNTHETIC ANIMATION

We therefore presented female *X. nigrensis* with synthetic animations of courting male conspecifics in the third experiment. Female *X. helleri* show a tenfold difference in response to animations of sworded versus unsworded males (Rosenthal & Evans 1998), suggesting that this method should be adequate for detecting sword preferences in *X. nigrensis*. We took two steps to address female motivation: (1) we tested females for a preference for the same synthetic male courting versus not courting, immediately before or after testing them for sword preference; (2) to standardize motivation, we randomly assigned females to one of two groups: in the first group we separated females from males for 30 days and then tested them, as above; we gave females in the other group oestradiol implants after the 30-day separation. Oestradiol is believed to regulate female sexual receptivity in at least one other poeciliid, the guppy *Poecilia reticulata* (Liley & Stacey 1983) and has been used to elicit responses to playback in birds (Searcy & Marler 1981).

Methods

Stimulus construction

We constructed a synthetic model of a courting swordtail as described in Rosenthal (2000b). All modelling, animation and rendering were done using 3D Studio Max 1.0 (Kinetix) on a Dell Optiplex GXPro computer, using a Targa 1000 board for digital-to-analogue conversion of video signals and vice versa. The animation represented a large genotype (*P-L*) male with average characteristics for that genotype, based on field measurements of sword extension length, standard length and body depth from 74 males from the Nacimiento Río Choy, Estación Hidrometrica, and a site further downstream at Las Palmas (22°0′51″ N, 98°52′17″ W). Fine-scale properties of the animated stimulus were derived from morphometric measurements taken from preserved specimens collected at the Nacimiento Río Choy between 1988 and 1993, in the Texas Natural History Collection, Texas Memorial Museum, Austin, Texas, U.S.A. We measured standard length and nine measures of body thickness using calipers ($N=17$). We recorded the position of 24 morphometric landmarks on each specimen using a dissecting microscope and a camera lucida ($N=19$).

We obtained outlines of body shape by placing preserved specimens ($N=19$) on an illuminated slide table to produce a high-contrast silhouette. We videotaped specimens for less than 1 s with a Hitachi VM-H100LA Hi-8 camera. We digitized a single frame per specimen with a Targa 1000 board in a Dell Optiplex computer, and used edge-finding routines in Adobe Photoshop (Adobe Systems) to produce a binary trace outline. We then aligned traces so that the snout–caudal peduncle axis was horizontal and scaled to match horizontal size across traces. We used the UTHSCSA ImageTool program (developed at the University of Texas Health Sciences Centre at San Antonio, Texas, U.S.A. and available from the Internet by anonymous FTP at maxrad6.uthscsa.edu)

to produce a grey-scale image in which each pixel reflected the mean grey value across corresponding pixels in each source image. We fitted by hand a single trace passing through the centre of the outline of the mean image, which we subsequently used as a source for the shape of the animated fish. We used the mean location of the appropriate landmarks to model both fin shape and location on the body for all objects except the sword extension, which we scaled to reflect the mean sword index for all large male *X. nigrensis* measured in the field.

Colour slides of male *X. nigrensis* were taken at the Nacimiento Río Choy within 30 min of capture, in a container designed for photography of aquatic organisms. We arbitrarily selected slides of individual males for use in providing texture parameters. We digitized the slides and isolated the morphological feature of interest (e.g. body, caudal fin, dorsal fin). We applied global colour corrections using Adobe Photoshop so that photographs would approximate colours as viewed on underwater videotape of animals in the field. Photographs were used to provide pattern and texture to the animation, except for the pelvic fins, gonopodium, caudal stripe and sword extension, whose small size and transparency made it unfeasible to use photographs directly. For these we estimated mean colour values from photographs using Photoshop; we modelled the gonopodium and pelvic fins with solid colours, whereas we used two contrasting colours for the inner and outer portion of the sword extension and caudal stripe.

The courtship behaviour of *X. nigrensis* shows qualitative differences between the laboratory and the field (G. G. Rosenthal & M. J. Ryan, unpublished data), making it unfeasible to quantify courtship behaviour in a controlled laboratory setting. Courtship involves the male positioning himself perpendicular to a female and executing a series of rapid turns (Ryan & Causey 1989). We videotaped sexually mature males in their habitat at the Nacimiento. We followed individual males for 5 min or until visual contact was lost. We measured sexual display length and interdisplay interval with frame analysis of videotape. We digitized 32 sexual displays, from at least 16 distinct individuals. We then estimated 15 motor pattern variables frame by frame. For variables that were unique to a given display, such as total display duration and number of turns during the display, we used the mean value from the 32 displays analysed. For variables that varied over the course of the animation, such as apparent speed and turn angle, we drew from a normal distribution within one standard deviation of the mean. The final animation consisted of a single looped sexual display. We adjusted the perspective of the screen view so that the view alternated between three different, orthogonal views of the same display. For the interval between courtship displays, we used the mean apparent speed measured from males between courtship bouts; the male swam in a straight line off screen and swam onscreen again at the start of the next presentation of the sexual display.

Females spend equal amounts of time viewing males against the homogeneous spacelight of the centre of the river as they do against the more heterogeneous substrate of rock, leaf litter, submerged wood and aquatic vegetation (G. G. Rosenthal, unpublished data), so we used a single background colour that reflected the mean colour value of 20 haphazardly selected point samples of spacelight taken from field videotapes. This ensured that the background we used was within the range of the modal background against which swords are viewed in nature.

This 'full' stimulus reflected mean values of the traits of interest: sword length and courtship behaviour. The sword index (sword extension length divided by standard length, Rauchenberger et al. 1990) was 0.17. For the 'no courtship' stimulus, we removed the courtship motor pattern and adjusted the swimming speed so that the male was visible on screen for the same amount of time as in the 'full' stimulus. For 'no sword' we removed the sword extension and caudal stripe, creating a stimulus similar to a swordless male in nature.

Manipulating female motivation

We randomly assigned females to one of two treatments: isolation for 30 days only (Morris et al. 1996) or isolation plus oestradiol implants. We anaesthetized fish assigned to the hormone treatment with clove oil and implanted them intraperitoneally with a 2×0.5 mm Silastic pellet treated with 0.02 mg/g oestradiol. We tested females 48–72 h after implantation. All females displayed normal feeding and association behaviour prior to testing. Because we were interested in evaluating alternative methodologies for standardizing female responsiveness, rather than isolating the effects of oestradiol, we did not attempt to control for the effects of handling and surgery.

Playback and data analysis

Females were drawn from Nacimiento and Estación Hidrometrica populations. Methods for playback were similar to those described in Rosenthal & Evans (1998). We tested females in a 76.8×31.8 cm aquarium filled to a height of 25.1 cm. The aquarium was divided into three 25.6-cm sections (left, right and centre) with a small clump of Java moss provided for cover in the centre section. A Panasonic TR-930B 9-inch (22.86 cm) monochrome monitor abutted either end of the aquarium. Monitor output was matched prior to each test by placing the monitors adjacent to each other, playing identical sequences, and then adjusting the controls until the images were indistinguishable. Presentation sequences were played on Panasonic AG-1970 S-VHS videocassette players. Subjects were acclimatized in the test tank with the monitors on for 10 min prior to the start of each test. We presented females with 10 min of monochromatic screen on both sides followed by simultaneous presentation of the 300-s test stimuli. The subjects were then presented with a 10-min monochromatic screen interval followed by the same set of stimuli presented on opposite sides of the set-up. We recorded the amount of time the female spent in each section for the duration of each stimulus period. We controlled for side biases across subjects by alternating the location of the first stimulus from one trial to the next.
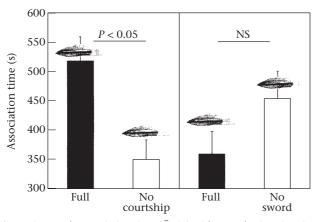
Figure 3. Female association time ($\bar{X}$+SE) with a synthetic animation of a courting, sworded male (■) versus the same animation with courtship or the sword removed (□). Illustrations taken from still images of animation presented to females.

We alternatively presented females with 'full' versus 'no courtship' followed by 'full' versus 'no sword', and vice versa. We tested each female once on these two stimulus pairs. We used a paired t test on log-transformed time scores to test the a priori hypotheses that females preferred the 'full' stimulus to 'no courtship' and that they preferred the 'full' stimulus to 'no sword'. We then compared the strength of preference (net time spent with 'full') for the two stimulus pairs. Finally, we used a two-factor analysis of variance (ANOVA) to examine effects of population and treatment (oestradiol-implanted versus nonimplanted) on strength of preference for the two traits.

Results

Females significantly preferred the full, courting stimulus to the same stimulus with courtship removed (paired t test: t_{36}=2.40, $P<0.02$; Fig. 3). Females failed to show a preference for this same 'full' stimulus over 'no sword', a courting male with the sword removed (t_{36}=1.04, two-tailed P=0.30; Fig. 3). As in experiment 2, females showed a tendency to prefer the swordless male. The preference for the full, courting, sworded male over the sworded, noncourting male was significantly stronger than that for the same stimulus over the swordless, courting male (t_{36}=2.91, $P<0.01$).

There was no effect of hormone treatment on preference for either stimulus pair (ANOVA: 'full' versus 'no courtship': $F_{1,36}$=0.07, P=0.80; 'full' versus 'no sword': $F_{1,36}$=0.09, P=0.77; N=37). We also failed to detect any effect of population ('full' versus 'no courtship': $F_{1,36}$=0.21, P=0.65; 'full' versus 'no sword': $F_{1,36}$=0.49, P=0.49).

Females displayed a preference for courtship when all other variables were controlled for, consistent with multivariate analyses of *X. nigrensis* preferences for male genotypes (Ryan et al. 1990) and with an experimental study with video playbacks on *X. helleri* (Rosenthal et al. 1996). The same females, however, failed to show a preference when the sword was removed from an identi-

cal stimulus. This result is unlikely to be due to low statistical power, given that the tendency for sword preference favoured the swordless male. We used two approaches to standardize motivation that produced no detectable difference in female behaviour. Females showed robust responses to variation in courtship, suggesting that they were in fact appropriately motivated to discriminate among potential mates. Finally, the contrast between the background and the sword reflected values found in nature, although these may have been misrepresented by the video output (see General Discussion).

Experiment 3 tested responses of females to swords of mean length. It may be, however, that predation or other forces have pushed mean sword length below the threshold required to elicit preference. This could also explain the apparently reduced internal contrast of *X. nigrensis* swords relative to congeners like *X. helleri*. In experiment 4, we tested female response to swords bearing extreme or supernormal characteristics.

EXPERIMENT 4: EXTREME AND SUPERNORMAL SYNTHETIC ANIMATION

Methods

We modified the 'full' stimulus from experiment 3 to construct an 'extreme' stimulus with a sword index of 0.45 (three standard deviations away from the mean), but within the range of natural variation (the maximum value measured was 0.56). We also modified the 'full' stimulus by increasing the height of the sword by 33% and providing a broad, yellow internal stripe, creating a '*helleri*'-like sword. Finally, we combined these two parameters to create an animation with an 'extreme *helleri*' sword. Females were tested on each of these stimuli against the 'no sword' stimulus from the previous experiment. To control for order effects, we varied the order of presentation for females that were tested on multiple stimulus pairs. Our previous results gave us no a priori prediction as to the direction of preference, so we used two-tailed Wilcoxon matched-pairs signed-ranks tests to evaluate preference for each stimulus pair. Methods were otherwise identical to the previous experiment.

Results

The tendency for all three stimulus pairs favoured the 'no sword' stimulus over exaggerated swords. Females did not show a preference for extreme over 'full' (Wilcoxon matched-pairs signed-ranks test: Z=1.02, N=28, P=0.31; Fig. 4a) or for '*helleri*' over 'full' (Z=0.97, N=14, P=0.33; Fig. 4b). Females significantly preferred 'no sword' over the extreme, *X. helleri*-like sword (Z=1.97, N=16, $P<0.05$; Fig. 4c).

GENERAL DISCUSSION

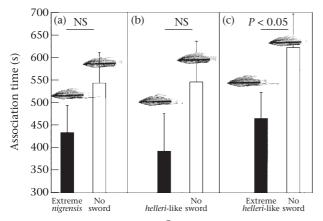Explaining the evolution of female mating preferences requires not only understanding how preferences evolve,

Figure 4. Female association time ($\bar{X}$+SE) with a synthetic animation of a male bearing no sword (□) versus identical stimuli with enhanced sword properties (■): (a) the length of the longest *X. nigrensis* swords; (b) the contrast properties of a *X. helleri*-like sword; or (c) a combination of the two. Illustrations taken from still images of animation presented to females.

but also understanding the conditions under which preferences are lost. To date, very few studies have documented an evolutionary reduction in the strength of female preferences. Demonstrating a preference reduction requires both sound evidence that a preference is currently not expressed in a taxon, and support for the hypothesis that a preference was present in an ancestor. Several studies have demonstrated interspecific variation in female preferences across species consistent with a scenario in which preferences were reduced (McClintock & Uetz 1996; Morris et al. 1996; Basolo 1998a), but in these cases it has been difficult to rule out the weaker preference as the ancestral condition.

Female *X. nigrensis* did not show a detectable preference for swords in any of our experiments. The seven experiments that we conducted used a total of 117 individual, responsive females from two populations, and we used various stimuli and experimental conditions. The conclusion that females show weak or no preference for sworded males is strengthened by the variety of conditions and methodologies we used. Furthermore, female sword preferences have been shown in other poeciliids using experimental approaches similar to those we employed (Basolo 1990a, b, 1995a, b, 1998a; Rosenthal & Evans 1998; Trainor & Basolo 2000).

It is possible that the lack of female preference in these experiments is in part due to the absence of ultraviolet (UV) cues in these trials. Ultraviolet light, although abundant during the day near the water surface, is effectively absent from most laboratory set-ups: most light sources do not emit in the ultraviolet, glass and acrylic dividers in aquaria are usually effective filters of UV light, and video and computer monitors do not emit directed UV light (I. Schlupp & K. Lunau, personal communication). The four species for which Basolo (1990a, b, 1995a, b) showed a sword preference were also tested under laboratory conditions with UV cues most likely absent. *Xiphophorus variatus* showed a preference for swords when presented with painted models

(Haines & Gould 1994) and *X. helleri* showed a strong preference for swords very similar to the animated stimuli in experiment 4 (Rosenthal & Evans 1998). The swords of *X. helleri*, like those of *X. nigrensis*, reflect in the ultraviolet (I. Schlupp & K. Lunau, unpublished data), yet females of *X. helleri* and other species demonstrate robust preferences for swords under UV-absent conditions (Basolo 1990a, b, 1995a, b, 1998a; Rosenthal & Evans 1998; Trainor & Basolo 2000).

Alternative phylogenetic hypotheses (Rauchenberger et al. 1990; Meyer et al. 1994; Borowsky et al. 1995; Marcus & McCune 1999) place *X. nigrensis* within a group of species with preferences for swords (*X. helleri*, Basolo 1990a; *X. maculatus*, Basolo 1990b; *X. variatus*, Basolo 1995a; and *Priapella olmecae*, Basolo 1995b). Our results strongly suggest that *X. nigrensis* females have secondarily lost the preference for at least the non-UV components of a sword, or for swords under nonultraviolet conditions.

Why has preference for swords been reduced? There is currently no evidence to suggest that either the sword or body size is an indicator of male genetic quality or direct benefits to females. However, if size is an honest indicator mechanism (Zahavi & Zahavi 1997), and swords act as an inexpensive (that is, dishonest) surrogate for apparent size (Rosenthal & Evans 1998), then selection may favour females who attend only to standard length in assessing size, since sword index is poorly correlated with body size. Swords may also induce females to mate at a rate higher than optimum or at inopportune times and places (Holland & Rice 1998). Alternatively, the density of *Astyanax mexicanus*, a major visual predator, is comparatively high in *X. nigrensis* habitat (Rosenthal 2000a; Rosenthal et al. 2001). *Astyanax mexicanus* are attracted to males with longer swords (Rosenthal et al. 2001). Predation may impose a cost on females of associating with males conspicuous to predators; such an association cost would have favoured a loss of preference for the sword, or even a bias against it. *Xiphophorus nigrensis* are not sympatric with any other swordtails, but both *X. multilineatus* and *X. pygmaeus* are sympatric with *X. cortezi*, whose males express broad, high-contrast swords. Selection may have favoured a bias against such swords as a means of avoiding hybridization with an *X. cortezi*-like ancestor. Finally, natural selection against swords due to predation may have caused a correlated reduction in female preference.

The maintenance of swords in *X. nigrensis* populations is puzzling given that predators find males with swords more conspicuous than swordless ones, and given the lack of a detectable female preference in this study. Differential predation on swords is likely to be sublethal (Rosenthal 2000a), so selection against swords may be relatively weak. If there is a genetic correlation between sword length and body size, courtship, or other sexually selected traits, the pleiotropic effects of sexual selection could maintain the sword in the absence of direct selection favouring the trait.

There has been little empirical work on the costs of female preferences. While some studies have shown that females change their preferences according to predation risk (Forsgren 1992; Berglund 1993; Hedrick & Dill 1993;

Godin & Briggs 1996; Rand et al. 1997), the probability of encountering heterospecifics (Pfennig 2000), or the operational sex ratio (Berglund 1994), few studies have shown that females with stronger preferences incur higher costs. Because of the evolutionary reduction in sword preferences in at least one swordtail species, these fish provide an excellent system in which to examine the effect of search and association costs on the evolution of female preferences.

Acknowledgments

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