

No pre-existing bias in sailfin molly females, *Poecilia latipinna*, for a sword in males

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Summary

The origination of female mate preference is still not well known and may depend on genetic predispositions, social environment and sensory stimuli in the environment. Females of different populations, which live in different environments, may therefore differ in a pre-existing bias for male traits. Previous studies within the genus *Xiphophorus* and *Priapella* (Poeciliidae) have indicated that females have a latent preference for a sword in males, even though conspecific males do not express a sword. In a recent study Basolo (2002a) found such a pre-existing bias for artificially sworded males in sailfin molly females from a Louisiana population. To investigate whether *Poecilia latipinna* females exhibit in general a pre-existing bias for sworded males or whether populations differ in a pre-existing female preference for sworded males, we tested *P. latipinna* females from a Texas population for a latent preference for sworded males. We tested in video playback experiments whether sailfin molly females *P. latipinna* have a latent preference for males with an artificial coloured plastic sword on TV monitors. Using video playbacks we first showed in a conditioning experiment that females perceived yellow plastic swords. Females preferred to associate with conspecific males to conspecific females on TV monitors. Females, however, did not exhibit a preference for males with a coloured sword over males with a transparent sword. Our result contradicts results of a previous study showing that sailfin molly females from a different population exhibited a preference for live males with an artificial sword. Pre-existing biases for novel male traits in females may differ between populations within a species. Thus, environmental factors may influence the development of pre-existing biases and might, therefore, drive the evolution of latent preferences in different populations differently.

Keywords: pre-existing bias, sailfin molly, sword, mate choice, video playbacks.

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Introduction

The evolution of mate preferences in females is one of the most fascinating processes in sexual selection. Theories of sexual selection provide different models to explain the origin, maintenance and extravagance of female mate preferences and how female mate choice forces the evolution of secondary sexual male traits (overview in Andersson, 1994; Jennions et al., 2001; Kokko et al., 2003). Female mate preference can co-evolve with the male trait in focus, as predicted by the indicator models (Zahavi, 1975; Møller & Alatalo, 1999) and arbitrary models (Fisher, 1930; Brooks, 2000), or female mate preference can evolve before a trait is expressed in males as stated by the sensory exploitation hypothesis (Ryan, 1990; Ryan & Keddy-Hector, 1992; Shaw, 1995; Endler & Basolo, 1998). In this scenario, females develop biases in mate preferences due to a sensory bias in their sensory systems formed by natural selection, sexual selection or pleiotropic effects. Females are preferentially attracted to males that stimulate their sensory systems more than other males. If males send a new signal, which matches the latent bias and stimulates the sensory systems in females, these males are preferred as mates. Thus, a pre-existing bias may affect the nature and direction of sexual selection when a new male trait arises. The sensory exploitation hypothesis has been tested in several different systems and taxa: in frogs: females of the species *Physalaemus coloradorum* show a strong preference for conspecific male calls with the heterospecific 'chuck' when added to the normal call with no 'chucks' (Ryan & Rand, 1993, 1995, 1999), in mites: females respond to vibrations on the water surface produced by prey and males exploit this response by vibrating their legs near a female (Proctor, 1992), in spiders: females of the tuft less wolf spider *Schizocosa rovnieri* prefer males with tufts added onto legs (McClintock & Uetz, 1996), in birds: females of the regent bowerbird *Sericulus chrysocephalus* show a preference for coloured food items which matches the preference for coloured bower decorations in males (Madden & Tanner, 2003). Female grackles *Quiscalus quisqualis* exhibit a preference for males singing four different song types, although conspecific males sing one song type only (Searcy, 1992). Females of three species of estrildid finches prefer mates with an artificial crest, although crests are lacking within this family (Burley & Symanski, 1998; Witte & Curio, 1999), in insects: females of three non-gift-giving cricket species accept novel food gifts from conspecific males and accept more sperm than they

would without the extra food (Sakaluk, 2000), in crabs: male fiddler crabs *Uca beebei* build mud pillars at their burrow entrances and exploit female use of vertical objects in orientation (Christy, 1995), and in fish species: guppy females prefer males with larger and more chromatic orange spots, and this mate preference originated from a preference for orange food items (Rodd et al., 2002).

In a group of live-bearing fishes, the Poeciliids (Rosen & Bailey, 1963), the role of pre-existing biases for the evolution of female preferences and male traits has been well studied. Within this family the subfamily Poeciliinae (Supertribe Poeciliini) contains seven tribes (Rauchenberger, 1989). Within the tribe Poeciliini, some species of the genus *Xiphophorus* possess a sexually dimorphic male trait, the sword. The sword is a composite trait consisting of an upper black stripe, a lower black stripe, internal carotenoid and/or pterin stripe coloration, and elongation of certain ventral caudal-fin rays (Basolo, 1998a). Swords have also UV components (Cummings et al., 2003). Males of the green swordtail *X. helleri* possess a typical sword with all these components. Female green swordtails prefer males with a longer sword to males with a shorter sword (Basolo, 1990a), thus the male sword is a meaningful trait in female mate choice. Not only females of the green swordtail exhibit a preference for males with swords (Basolo, 1998a, 1998b) but also females of *X. maculatus* and females of *X. variatus* prefer conspecific males with a surgically attached sword to swordless males, although conspecific males never express a sword in nature (Basolo, 1990b, 1995a). This female preference for swords in males is also expressed in females of the group *Priapella*, a sister genus of the genus *Xiphophorus* (Rosen, 1979; Rauchenberger et al., 1990; Meyer et al., 1994), although *Priapella* males never express swords (Basolo, 1995b).

In general, female mate preference for expressed male traits is determined by genetic, social or environmental factors, or a combination of two or even three factors (overview in Andersson, 1994). Female mate preference can be stable over an evolutionary long time, even when the male trait is absent or reduced (Houde & Hanks, 1997). Female mate preference can also be lost due to extrinsic factors. Rosenthal et al. (2002) discovered a secondary loss of a preference for sworded males in *X. nigrensis* females. They argued that a secondary loss is favoured by selection if a preference for sworded males would cause females to mate at a suboptimal rate, risk mating with heterospecifics, or mate with phenotypically or genetically inferior mates.

The origination of female mate preferences can be influenced by such extrinsic factors as well. The development of a pre-existing bias in mate preference may depend on genetic predispositions, social environment and sensory stimuli in the environment and may change over time. Thus, a pre-existing bias in mate preferences is not necessarily evolutionary fixed. Females of *P. olmecae* exhibit a significantly stronger preference for a sword in males than females of *X. helleri*, although *P. olmecae* males never express a sword (Basolo, 1998a). Pre-existing biases may depend on environmental factors and may, therefore, differ between different populations within a species. In a recent study, Basolo (2002a) found a female preference for males with a sword in the sailfin molly *P. latipinna* from a Louisiana population. To investigate whether *P. latipinna* females exhibit in general a pre-existing bias for sworded males or whether populations differ in a pre-existing female preference for sworded males, we tested *P. latipinna* females from a Texas population for a latent preference for sworded males.

We investigated whether sailfin molly females showed a preference for conspecific males with an artificial sword imitating the sword of the green swordtail *X. helleri*. Sailfin molly males never express sword like elongations of the tail fin.

We performed mate choice experiments by using video technique instead of presenting live stimuli. To ensure that video presentation is a useful technique in our study species, we first tested in a conditioning experiment whether females were able to perceive a coloured plastic sword in front of a green background on TV monitors as used in mate choice experiments. We then tested whether females were able to distinguish between a conspecific male and a conspecific female on TV monitors. Thereafter we performed a mate choice experiment testing a preference of females for sworded males.

Methods

Sailfin mollies are live-bearing poeciliid fishes without parental care. They live in mixed-sex shoals comprising 10-20 individuals (Witte & Ryan, 2002). All fish used in the experiments were at least 6 months old, sexually experienced, and descendants of wild fishes from the Comal River near New Braunfels, Texas, U.S.A., caught in summer 1999. We maintained the fish separated by sex in several tanks (100 × 50 × 50 cm) under a 12:12 h

light : dark cycle with broad spectrum fluorescent light (Osram FLUORA, L58 W/77), at an average temperature of 24°C, and fed them ad libitum with flake food (Tetramin), chironomid larvae, *Daphnia* spec. and/or *Artemia* nauplii once a day. We performed experiments between July and October 2001.

Conditioning experiment

For the mate choice experiments with video presentations it was essential to know whether females can perceive yellow artificial swords in front of a green background which was used in mate choice experiments (see below). We therefore performed a conditioning experiment to investigate this question. In this conditioning experiment we tested whether females can learn to associate food with yellow swords fixed on fishing lines. If so, this would indicate that females could perceive yellow swords on the TV monitor.

Artificial swords

We cut sword-like pieces out of a yellow plastic sheet. The swords had the typical shape of a natural sword of swordtail males *X. helleri* with the largest width of 4 mm at the basis and tapered to the tip. All swords were 2.5 cm long. To imitate the sword of *X. helleri* males, we draw an upper black stripe and a lower black stripe with a permanent waterproof black pen (Edding). We also created transparent swords and cut pieces of the same shape and same size like the yellow swords out of a transparent plastic sheet. The plastic swords we used in the conditioning experiment had a similar length and the same shape as the plastic swords we used in the mate choice experiments. The yellow and transparent plastic swords showed no reflectance under UV.

Preparation of video stimuli

We attached two yellow plastic swords at different height to each of 10 fishing lines in a recording tank (50 × 50 × 50 cm height). The fishing lines were vertically fixed at the ground and top of the recording tank, so that 20 swords floated horizontally and equally distributed in the recording tank. The recording tank was filled with water, had a layer of gravel and a green paper sheet as a background (same paper sheet as used in the video presentation in the mate choice experiments). We recorded the yellow swords with a professional-quality Panasonic S-VHS-C Movie Camera (NV-VS4 E6/E) on high-quality S-VHS Fuji tapes (PAL video standard) from 1 m distance for

45 min. We also recorded 20 transparent plastic swords attached to fishing lines in the same manner for 45 min. The fishing lines and the transparent swords were not visible to us. Additionally, we recorded 10 fishing lines with no swords fixed on in front of a green background for 45 min.

Learning period

We kept two groups of 11 females each in a tank ($50 \times 25 \times 30$ cm high). Both tanks stood side by side with an opaque partition in between. Both tanks were illuminated from above with broad-spectrum fluorescent light (Osram FLUORA, L58 W/77). The water temperature was 24°C . A TV monitor 51 cm in diagonal (SABA, M5520C, 50 Hz, Type 630Z/TX91 GEU) stood 1 cm in front of each tank. Each monitor was connected to a JVC Super-VHS video recorder (HR-S 7500 E/EH). For a period of 14 days we fed the females twice a day with flake food, chironomid larvae, *Daphnia* spec. and *Artemia salina*. Each time when we fed the fish we played the videos for 45 min. To one female group we always presented yellow swords, to the other group we always presented transparent swords during feeding. After the 45 min presentation we covered the TV monitors and removed the food that was left after 45 min. After this two weeks learning period we tested females in a binary choice situation whether they have learned to associate a positive stimulus (food) with yellow swords or transparent swords or not.

Learning test

In the binary choice tests we tested females in a test tank ($50 \times 50 \times 50$ cm height) with a TV monitor (same as used in the learning period) on each side. The test tank had a layer of gravel on the ground and was filled up with water at 24°C . The test tank was partitioned into three zones, i.e. two preference zones at each end of the tank and a neutral zone in between. Each preference zone was marked by a vertical black line on the front and back surface 10 cm from each end of the tank. Females, which we had presented yellow swords during the learning period, were given a choice between a video showing yellow swords on one side and a video showing transparent swords on the other side of the test tank. Females of the other group, which we had presented transparent swords, were given a choice between a video showing transparent swords and a video showing only fishing lines. The side for presenting the three different videos was randomised.

Before a test started, a test female had 20 min to acclimate to the test tank. During this period the video monitors were covered with white plastic boards and the female moved freely within the tank. After the female had acclimated to the test tank, we gently placed the female in a clear Plexiglas cylinder (11 cm diameter) in the centre of the test tank, started the videos, removed the white plastic boards and presented the videos on both sides for 2 min. Thereafter we gently released the female out of the cylinder and measured the first response of the female, that is which preference zone she entered first. A test lasted a maximum of 10 min. A female that did not enter one of the two preference zones within 10 min was omitted from the analysis. Females used in the conditioning experiment were not used in mate choice experiments.

Mate choice experiments

The artificial sword

All yellow and transparent swords were produced as described above. Swords had a length of 50% of the standard body length of the male to whom the plastic sword was attached. The width of the sword was 33% of the width at the base of the tail fin of the male. For glueing the transparent or yellow sword onto the tail fin we gently placed a male in a 0.2% solution of urethane for 5-20 min. After the male was anaesthetized we laid the male on a wet paper sheet and covered all parts of his body except the tail fin with wet paper sheets. We glued the plastic sword on the basal part of the tail fin with a drop of cyanacrylate (UHU-Sekundenkleber). After the manipulation we gently released the male in the recording tank (see below) where he could recover for at least 30 min. There were no indications for lasting effects of anaesthesia and we observed no adverse effect of gluing the sword onto the tail fin in males. The coloured sword was clearly visible to us on the monitors; the transparent sword was not visible to us on the videos.

Preparation of video stimuli

All videos were recorded with a professional-quality Panasonic S-VHS-C Movie Camera (NV-VS4 E6/E) on high-quality S-VHS Fuji tapes (PAL video standard). The fish was recorded in a recording tank (50 × 30 × 30 cm height) filled with water with the longest side facing the camera. The camera was placed in 1 m distance from the recording tank. The tank had a green

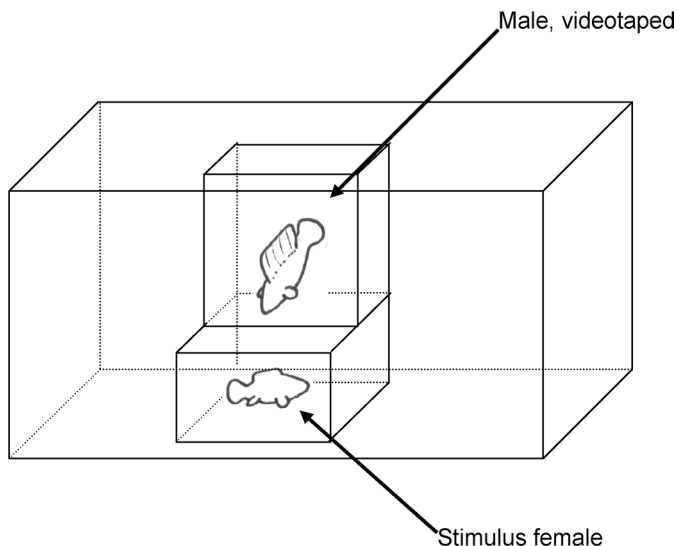


Figure 1. Side view of the recording tank. The stimulus female was in the lower tank and was not recorded, the male, here with no sword, was in the upper tank and was recorded in 1.5 m distance.

paper sheet as a background and was illuminated from above with broad-spectrum fluorescent light (Osram FLUORA, L58 W/77). To record courting males we used the recording design of Rosenthal et al. (1996). We placed a female within a small tank ($25 \times 10 \times 15$ cm) upside down on the bottom of the recording tank (Figure 1). We placed a male in a smaller tank ($15 \times 10 \times 15$ cm) on the rear part of the female tank. The male courted the female underneath through the glass bottom, facing the camera, so that his courtship displays appeared to be directed towards the test female viewing the videos in the experiment. We recorded only the male, not the female. For recording single females we placed a female in the top tank and removed the female in the lower tank.

To create good stimuli for test females all fish were played back 1.7 times larger than their natural body length. The apparent body length of the stimulus fish on the video monitors were still within the natural range of standard body length (up to 100 mm total length, Sterba, 1978) for sailfin mollies. We created four sets of videos: a single male with no sword, a single female, a single male with a yellow sword, and a single male with a transparent sword. Each video lasted 11 min for experiments 1 and 2, and 16 min for the second experiment.

For Experiment 1 in which we tested whether females could distinguish between a female and a male on the TV monitor, we recorded three single females (natural standard body length: 37, 38 and 40 mm) and three single males (37, 39 and 40 mm). In Experiment 2 we tested whether the manipulation had any effect on male behaviour. We recorded three other single males (34, 35 and 37 mm) twice, each male with no sword and with a transparent plastic sword. For the third experiment we recorded eight males (32, 33, 34, 35, 42, 42, 43 and 44 mm) twice, each male with a transparent plastic sword and with a coloured plastic sword. After recording a male we removed the plastic sword without damaging the tail fin. All fish were maintained in our stock population after the experiments.

General procedure

All experiments were conducted in an aquarium (100 × 50 × 40 cm height) with natural gravel on the ground, water temperature of 24°C, and with a video monitor of 51 cm in diagonal (SABA, M5520C, 50 Hz, Type 630Z/TX91 GEU) at each side of the tank. The aquarium was not illuminated from above to avoid any light reflections on TV monitors. Each monitor was connected to a JVC Super-VHS video recorder (HR-S 7500 E/EH). The test tank was partitioned into three zones, i.e. two preference zones at each end of the tank and a neutral zone in between. Each preference zone was marked by a vertical black line on the front and back surface 20 cm from each end of the tank.

Before a test started, a test female had 20 min to acclimate to the test tank. During this period the video monitors were covered with white plastic boards and the female moved freely within the tank. After the female had acclimated to the test tank, we gently placed the female in a clear Plexiglas cylinder (11 cm diameter) in the centre of the test tank, removed the white plastic boards and started the videos on both sides simultaneously. After four min (after 10 min in the third experiment), monitors were covered and the videos rewound and restarted. After this we removed the cover and released the test female out of the cylinder and measured the time she spent within the preference zones in front of the videos for the next 10 min. Then we covered the video monitors, placed the test female back into the cylinder, switched and rewound the videos, removed the white plastic boards, played the videos, released the female into the test tank and measured the time she spent within the preference zones within the next 10 min again. A female

was considered to have chosen a stimulus fish when she spent more than 50% of the total time she spent in both preference zones in front of that fish during the 2×10 min test periods. Although time spent is an indirect measure of female mate preference, Bischoff et al. (1985) and Kodric-Brown (1993) have shown in guppies that the time females spent together with a male correlates positively with the probability of copulation with that male. This was found in other species as well (Ryan et al., 1990; Forsgren, 1992; Berglund, 1993). In the sailfin molly, female association time with a male in a situation with no physical contact predicts the probability of copulation with that male (Witte, unpubl. data).

After each test, we measured the standard body length (from the tip of the snout to the end of the caudal peduncle) of each female with callipers. We did this after the experiment because this procedure was stressful for the fish. All tests were observed from behind a black curtain behind. Before testing test females were kept in separate tanks. Test females had no experience with the stimulus males used in the tests prior to testing.

Experiment 1: male versus female

In this experiment we tested whether females were able to distinguish between a conspecific male and a conspecific female on the video monitor in a binary choice situation. We randomly presented a video showing a male on one side and a video showing a female on the other side of the test tank. After the female had acclimated to the test tank, we measured the time she spent within the preference zone in front of the male video and in front of the female video for the next 2×10 min trials as described above. We combined one of the three female videos with one of the three male videos and used each of nine combinations a balanced number of times. We tested 128 females with an average standard body length (+ SD) of 35.7 ± 4.1 mm.

Experiment 2: no sword versus transparent sword

Here we tested whether the manipulation, i.e. glueing the plastic sword onto the caudal fin, had any effect on male behaviour like swimming activity and mobility which might decrease the attractiveness of males to females. Females could choose between a male with no sword on one monitor and the same male with a transparent sword on the other monitor. Thus, males presented in a test differed only in having a transparent sword or not. We

used all three male combinations a balanced number of times. We tested 105 females with an average standard body length (+ SD) of 35.8 ± 4.3 mm.

Experiment 3: Transparent sword versus coloured sword

In this experiment females could choose between a male with a transparent sword and the same male with the coloured sword. Before the female could choose between a male with a coloured sword on one side and the same with a transparent sword on the other side, the female was within the clear Plexiglas cylinder for 10 min and could already observe the male stimuli. We used all eight male combinations a balanced number of times and tested 60 females with an average standard body length (+ SD) of 36.7 ± 4.8 mm.

Data analysis

In the conditioning experiment, we used a binomial test to analyse the number of tests in which females entered one of two preference zones first. In the mate choice experiments, we used a binomial test to analyse the number of tests in which one of both video stimuli was preferred by the test female, i.e. the test female had spent more than 50% of the time she had spent in both preference zones in front of that stimulus during the 2×10 min of the preference test. We used a Wilcoxon signed-ranks test to analyse the actual time test females spent in both preference zones during a preference test, and to analyse whether females choose consistently between the first and second part of a choice test. We used a power analysis to calculate the power of tests (t-test of dependent samples) and for calculating the sample size to receive significant results based on our data.

Results

Conditioning experiment

Ten out of 11 females that we had presented yellow swords on videos during the learning period entered the preference zone in front of the yellow swords first. One female swam into the preference zone in front of transparent swords first (binomial test: $N = 11$, $p = 0.006$). Thus, we conclude that these females have associated a positive experience with yellow swords and, therefore, preferred to swim towards this stimulus in expectation of food.

This response is only possible when females can perceive yellow swords. Thus, this experiment provides good evidence that sailfin molly females are able to see yellow swords on a green background. Females that we had presented transparent swords during the 2-week learning period showed no clear preference for the video showing transparent swords or the video showing fishing lines only. Six females entered the preference zone in front of transparent swords first and four females entered the preference zone in front of fishing lines first (binomial test: $N = 10$, $p = 0.37$). One female did not enter one of the preference zones within 10 min and was omitted from the analysis. This result could indicate that these females either did not perceive the transparent swords or that they have associated the green background which was identical for both presentations with food. If latter is the case, this means that the green background was a better stimulus to perceive than transparent swords for females. The clear preference of females that have been presented with yellow swords show that females can definitely see yellow swords on a green background and that transparent swords are a very weak or no stimulus to females.

Experiment 1: male versus female

Test females showed a clear preference for the male video (binomial test: $N = 128$, $p < 0.001$). Females spent on average 435.4 ± 242.4 s ($= 57.3 \pm 22.5\%$) in front of the male video and only 325.6 ± 214.8 s ($= 42.7 \pm 22.5\%$) in front of female videos (Wilcoxon signed-ranks test: $z = 3.28$, $N = 128$, $p < 0.001$, Figure 2A). Females chose consistently between the first and second part of preference tests (Wilcoxon signed-ranks test: $z = -0.33$, $N = 128$, $p = 0.74$). Thus, females could distinguish between a conspecific male and a female on TV monitors.

Experiment 2: no sword versus transparent sword

Females chose consistently between the first and second part of preference tests (Wilcoxon signed-ranks test: $N = 105$, $z = -0.9$, $p = 0.36$). Test females did not discriminate between a given male with no sword and the same male with a transparent sword (binomial test: $N = 105$, $p = 1.0$). Females spent a similar amount of time in front of the male in both conditions: males with no sword: 363 ± 235.5 s ($= 49.6 \pm 24.3\%$) and males with a transparent sword: 375.4 ± 244.3 s ($= 50.4 \pm 24.3\%$) (Wilcoxon signed-ranks test:

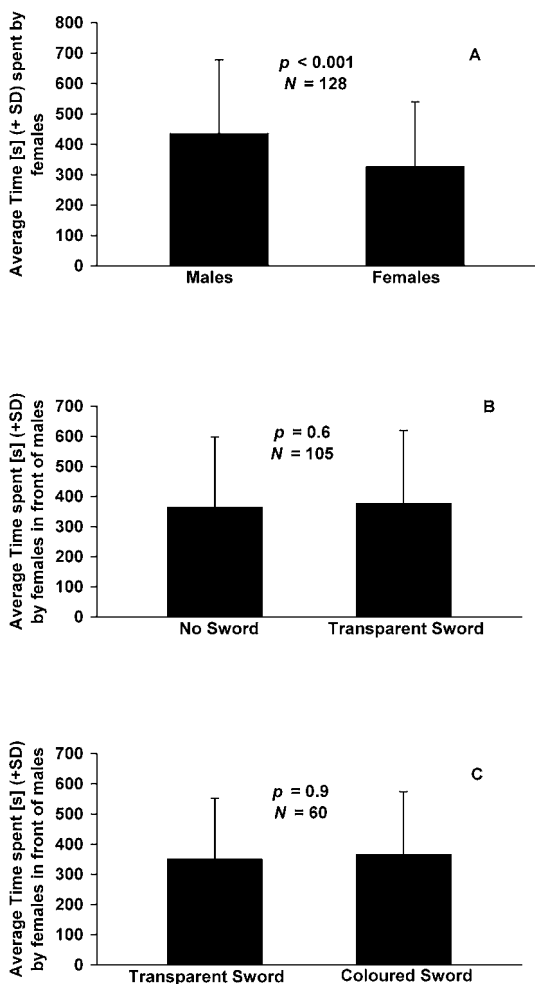


Figure 2. A — Experiment 1: Average time a female spent (mean + SD) in front of a male video and a female video. B — Experiment 2: Average time a female spent (mean + SD) in front of a video showing a male with no sword and a video showing a male with a transparent sword. C — Experiment 3: Average time a female spent (mean + SD) in front of a video showing a male with a transparent sword and a video showing a male with a coloured sword.

$N = 105$, $z = 0.54$, $p = 0.6$, Figure 2B). A power analysis revealed that if sailfin molly females spent only 0.8% more of their time in front of males with a transparent sword, we would need a sample size of 7342 to reject the null hypothesis. We conclude that the manipulation did not decrease the attractiveness of males to females.

Experiment 3: Transparent sword versus coloured sword

Females chose consistently between the first and second part of preference tests (Wilcoxon signed-ranks test: $N = 60$, $z = -1.5$, $p = 0.13$). Females showed no preference for a given male with a coloured sword or with a transparent sword (binomial test: $N = 60$, $p = 0.9$) and spent a similar amount of time in front of the male with a transparent sword (349.6 ± 202.5 s, $= 49.3 \pm 21.5\%$) and with a coloured sword (364.9 ± 208.8 s, $= 50.7 \pm 21.5\%$) (Wilcoxon signed-ranks test: $z = 0.99$, $N = 60$, $p = 0.9$, Figure 2C). A power analysis revealed that if sailfin molly females spent only 1.4% more of their time in front of males with a coloured sword, we would need a sample size of 3518 to reject the null hypothesis. We, therefore, conclude that sailfin molly females of our study population showed no pre-existing bias for males with a coloured sword.

Discussion

Our study provides no support for a pre-existing bias in sailfin molly females for a sword in conspecific males in the Texas population. Females of the Louisiana population (Basolo, 2002a) and our Texas females remarkably differ in the pre-existing bias for sworded males. Our negative result might be explained due to methodical factors or by population differences due to different environmental conditions. We will discuss first possible methodical factors and then discuss biological reasons for this difference in female mate preference. First, it could be that females did not perceive a conspecific male on the TV monitors because TV monitors are designed for the human visual system and video stimuli might be perceived differently by different species (Fleishman et al., 1998; Fleishman & Endler, 2000; Oliveira et al., 2000 for a review), and species differ in the sensitivity and distribution of photoreceptors (Lythgoe & Partridge, 1989). Due to different properties of the visual systems especially the colour perception might be a major problem (Oliveira et al., 2000). However, if the images are presented at a high frame rate and the pixel spacing of the monitors is sufficiently small, most animals should be able to use the visual cues presented in playback studies properly. The result of our first experiment showed that sailfin molly females were able to distinguish between a conspecific male and a conspecific female on the video monitor. This result validates our method using video playbacks instead of

live stimulus fish in the mate choice test as well. In a previous study using video technique (Witte & Ueding, 2003), sailfin molly females distinguished between conspecific males and females as well and these authors found a similar strong preference for males over females as we did in this study. Trainor & Basolo (2000) showed in green swordtails *X. helleri* that females could distinguish between a conspecific male and a female on a monitor. Testing the ability of fish to discriminate between the sexes is an important test for using video playbacks in mate choice experiments. Alternatively, a test of species recognition can be performed as well. Thus, using male video instead of live males did not cause the lack of finding a pre-existing bias for sworded males in sailfin molly females.

Second, it could be that females were able to perceive conspecific males, but did not see the yellow black-bordered swords attached to males. The results of the conditioning experiment, however, indicated that sailfin molly females learned to associate food with yellow plastic swords in front of a green background. This learning process requires that females can perceive yellow swords on the TV monitor. We conclude, therefore, that females were also able to perceive yellow swords attached to males shown on the video presentations in the mate choice experiment. A conditioning experiment is an excellent method to investigate whether a test fish can perceive a stimulus in focus in a video experiment.

Third, it could be that females perceived the plastic sword attached to males, but need to see UV components of a sword to recognise a sword as a natural male trait. The plastic swords we used showed no UV reflectance and due to the TV monitors and glass tanks, no UV could be transmitted to test females. Although the natural sword in male swordtails contains UV components (Cummings et al., 2003), swordtail females are able to recognise conspecific males and demonstrate strong mate preferences when UV was eliminated via glass using live males (Ryan et al., 1990) or videos (Rosenthal et al., 2002). We, therefore, conclude that lacking UV components due to TV monitors or no UV-reflectance of plastic swords would not hinder to detect a pre-existing preference in females for sworded males if there was one.

Fourth, it could be that females were not motivated to choose between males. Studies comparing female response to live males and males on video monitors showed, however, that females spent a similar amount of time in front of a male video and a live male behind a glass: in guppies (*Poecilia reticulata*) (Kodric-Brown & Nicoletto, 1997), in green swordtails (Trainor

& Basolo, 2000), and in *P. formosa* (Gonçalves et al., 2000). Females of *X. cortezi* showed even a higher repeatability in mate preferences when video animations were presented in a choice situation than when live males were presented as stimuli (Morris et al., 2003). Körner et al. (1999) and Landmann et al. (1999) successfully used video technique in mate choice tests with *P. formosa*. Thus, video stimuli are adequate stimuli in Poeciliids and provide an excellent design for mate choice experiments. In our study females responded properly in the situation when they were presented a male and a female video. And they spent significantly more time within the preference zone in front of the male video than in front of the female video. Additionally, test females behaved similar to other sailfin molly females, which we have tested in binary choice situations with live stimulus males in previous experiments (Witte & Ryan, 1998; Witte & Noltemeier, 2002). Thus, females were highly sexually motivated during experiments. We, therefore, think that it is very unlikely that using video males causes the lack of a pre-existing bias for sworded males in our female population.

The lack of a pre-existing preference for sworded males in sailfin molly females used in our experiment could not be explained by methodical reasons. Why do sailfin molly females differ in a pre-existing bias for sworded males in the Louisiana (Basolo, 2002a) and Texas population? It is well known that guppy females (*P. reticulata*) from different populations exhibit different mate preferences regarding existing male traits (Houde, 1997), and that the expression of a male trait sometimes does not correlate with the female's preference. Guppy females from the Yarra River exhibit a strong preference for more orange males, although males of the Yarra River express less orange than males of other populations (Houde & Hankes, 1997). Such a population difference could exist for pre-existing biases as well. Females of different populations live in different environments, which can influence the sensory system in females differently and lead to differences in sensory biases in females. Basolo (2002a) investigated the preference for sworded males in two *Poecilia* species, in the sailfin molly *P. latipinna* from a Louisiana population and in the Atlantic molly *P. mexicana*. Females of *P. latipinna* preferred males with a coloured plastic sword to males with a transparent plastic sword. Females of *P. mexicana*, however, did not discriminate between these two male types. It remains unclear whether *P. mexicana* females have no pre-existing preference for sworded males evolved, or whether females of this species have secondarily lost such a pre-existing preference. The same

argument may be applicable to explain the different outcome of Basolo's (2002a) and our study. It could be that sailfin molly females from the Texas population have no pre-existing bias or have secondarily lost a pre-existing bias for sworded males before that trait was expressed in males.

Basolo (2002a) and we used females from different populations, which also live in different environments. Females of the Louisiana population used by Basolo lives in totally murky water (Basolo, pers. com.). Females from the Texas population live in crystal clear water in the Comal River. The visibility in water can have a tremendous influence on performing mate preferences and expression of male traits. In murky water the predation pressure is less strong than in clear water (Abrahams & Kattenfeld, 1997). Thus, it could be too costly for sailfin molly males to develop a sword in clear water and too costly for females preferring such a male type due to high pressure of predators, whereas in murky water bearing a sword and expressing a preference for sworded males are less costly and, therefore, a preference for swords is expressed in females of the Louisiana population. Another difference between murky and clear water is the visibility between conspecifics. Franck et al. (2001) showed that females of the green swordtail spent more time with males having black spots than with non-spotted males under murky water conditions, but not under clear water conditions. They argue, that females could better perceive males with black spots than those without black spots in murky water. A sword in *P. latipinna* males may increase the visibility of males for females and probably enhance their mating success in murky water. In clear water, however, a sword may not or only marginally increase the visibility of males for females.

In a recent study, Basolo (2002b) tested *Heterandria bimaculata* females for a pre-existing bias for sworded males. She found that these females discriminated against artificially sworded conspecific males. Females spent significantly less time in front of sworded males than in front of males bearing a transparent plastic sword. *H. bimaculata* is a member of another tribe within the Poeciliidae (tribe: Heterandriini). Basolo argues that *H. bimaculata* females discriminated against sworded males, because this species co-occurs with *X. helleri* in nature. Thus, females lower the risk of mating with the wrong species by discriminating against males with a sword. Sailfin molly females in our Texas population, however, did not co-exist with another poeciliid fish bearing a sword. Thus, this argument cannot explain the lack of a pre-existing bias in the Texas population.

In most studies, a pre-existing bias for novel traits has only been tested in a single population within a species. Populations, however, can differ in preferences for novel traits. For a more general understanding of the evolution of pre-existing preferences for novel traits it is important to test different populations of the same species. The impact of environmental factors on developing a pre-existing bias in females should be more considered in future studies to understand the complex process of the evolution of female mate preferences.

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