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RESEARCH PAPER

Do Female Western Mosquitofish, *Gambusia affinis*, Prefer Ornaments That Males Lack?

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Abstract

Some species in the family Poeciliidae are known for extravagant male ornaments and courtship behavior (e.g., guppies), but the majority of poeciliids are characterized by coercive male copulation attempts that seem to circumvent female choice. In some lineages with male ornaments, female sensory bias may have preceded the evolution of corresponding male signals. We examined female preferences for colorful ornaments in Western mosquitofish, *Gambusia affinis*, in which males lack ornamentation and reproduce primarily through coercive mating attempts. We found that females exhibited a positional affinity for males that were artificially ornamented with blue coloration over males that had been treated with a transparent ornament. Females exhibited the opposite effect for males treated with red ornaments. In contrast, focal females did not exhibit behavioral discrimination between two live stimulus females or two models (silver fishing lures) with blue vs. transparent ornaments. This suggests a sexual context for female discrimination between males based on ornament color and whether an ornament was present. Because tribe Gambusiini is the basal branch of family Poeciliidae, the results of this study are consistent with the hypothesis that female responsiveness to male coloration is the ancestral poeciliid character state.

Introduction

The evolutionary tug-of-war between traits that increase survivorship and those associated with sexual attraction has been the subject of much attention since Charles Darwin introduced his theory of sexual selection (Darwin 1871; Fisher 1930; Hamilton & Zuk 1982; Parker 2006). Many animal species, from peafowl (Petrie 1994) to sticklebacks (Rowland 1994), exhibit costly male behavior and ornaments that are associated with corresponding female mating preferences. The interaction between sexual selection and other forms of natural selection is therefore particularly interesting when females exhibit mating preferences for characteristics that males do not have.

A mismatch between female preference and male signal is apparent when congeneric females have similar mating preferences, but males of some species lack the corresponding signal. For example, Basolo (1995) proposed that the sword-shaped male tail in some species of the poeciliid genus *Xiphophorus* evolved in concert with a preexisting female bias for this shape. In swordless congeners (platyfish), females prefer males with experimentally affixed prosthetic swords. Some phylogenetic analyses of the genus suggest that the female preference for swords evolutionarily preceded the appearance of the male signal (Rosen 1979; Rauchenberger et al. 1990; Basolo 1990, 1991; but see Meyer et al. 1994). Indeed, the preference for swords extends into other related, but swordless, genera (Basolo 1995).

Given this sensory exploitation hypothesis (see Ryan 1990) that preexisting sensory biases in females drove the evolution of male secondary sexual characters in *Xiphophorus*, we were curious about the relative lack of male ornamentation in other poeciliid lineages. Beyond *Xiphophorus* and *Poecilia* (guppies), male sexual ornaments are relatively rare in the family Poeciliidae, where lack of male courtship and ornaments appears to be the ancestral state (Bisazza

et al. 1997). Whether female preferences for male ornaments are ancestral or derived within the Poeciliidae, however, is currently unknown. If female ornament preference is the ancestral state, we might expect it to appear in mosquitofish (*Gambusia sp*.) as tribe Gambusiini appears to be the basal branch of subfamily Poeciliinae (Bisazza et al. 1997).

Mosquitofish are widespread and abundant live-bearing poeciliids, found on almost all continents (Krumholz 1948; Lloyd & Tomasov 1985; Lloyd 1986; Pyke 2005). The sexes are dimorphic in shape and size, with females larger than males and lacking the modified anal fin (gonopodium) that serves as an intromittent organ. However, mosquitofish males typically differ little from females in coloration [a notable exception is the Bahamas mosquitofish, *Gambusia hubbsi*, in which males exhibit orange dorsal coloration (Martin et al. 2014; Heinen-Kay et al. 2015)]. It is hypothesized and supported that intersexual selection for male ornaments is driven by female choice in multiple poeciliid lineages (e.g., guppies: Endler 1983; Houde 1997; swordtails: Basolo 1990). Therefore, it seems possible that female preferences evolved before the basal Gambusiini diverged from other poeciliine tribes. We predict that female mosquitofish may exhibit preferences for ornaments even in species with no corresponding male signals.

Males of most mosquitofish species do not appear to use colorful or elaborate traits to attract females. Instead, males are coercive and seem largely devoted to sexual pursuit and harassment of females with attempts at insemination through gonopodial 'thrusts' at female urogenital openings (Haynes 1993; Pilastro et al. 1997). Coercive copulation strategies may have evolved intrasexually under sperm competition, as females store sperm and each brood can have multiple sires (Constantz 1984). Male mosquitofish with larger bodies are known to chase and preclude smaller males from proximity to females (Itzkowitz 1971; Bisazza and Marin 1995), and there is evidence that females prefer to mate, or at least associate with, larger males (Hughes 1985; Bisazza et al. 2001). However, small males are more likely to secure successful copulations, presumably because they are less detectible by females and have better maneuverability for inserting the gonopodium into the female reproductive tract (Bisazza & Marin 1995; Bisazza & Pilastro 1997; Bisazza et al. 2001).

Intrasexual selection among males can lead to an evolutionary sexual conflict that negatively influences the fitness of females (Chapman et al. 2003; Hosken & Stockley 2005). In mosquitofish, the lack of courtship behavior, the intensity of sexual coercion by

males, and the mismatch between female association with larger males but high copulatory efficiency of smaller males suggest that male sexual behavior can negatively influence female reproductive success. For example, female mosquitofish spend inordinate amounts of time avoiding or fleeing coercive males (Pilastro et al. 1997), which can lessen female foraging efficiency by half (Pilastro et al. 2003) and presumably reduce the number and size of eggs produced. Heinen et al. (2013) found that male *G. hubbsi* in populations with predators chased females more frequently. Predators appear to drive selection that favors mating early and often. Female *G. holbrooki* mitigate male harassment and improve foraging efficiency by shoaling with groups of conspecific females (Bisazza et al. 2001; Pilastro et al. 2003).

Sexual selection appears to operate differently among poeciliid lineages, with intersexual selection stronger in guppies (*Poecilia*) and swordtails (*Xiphophorus*), but intrasexual selection generally prevailing in mosquitofish (*Gambusia*, but see Heinen-Kay et al. (2015) for a recent study of sexually dimorphic *G. hubbsi*). This diversity of mating systems among the Poeciliidae suggests that female preferences might be more widely distributed among taxa than corresponding male signals. Bisazza et al. (1997) conducted a phylogenetic analysis of the Poeciliidae using sequence data from the large (16S) mitochondrial ribosomal gene and reached two interesting conclusions. First, the Tribe Gambusiini appears to be the basal branch in the clade, so lack of male ornamentation is probably the ancestral character state. Second, male ornaments and courtship behavior likely evolved independently in *Poecilia* and *Xiphophorus*.

If ancestral poeciliid males lacked sexual ornaments, a preference for ornaments by contemporary female *Gambusia* would be consistent with a sensory exploitation hypothesis that preexisting female biases drove the evolution of male ornamentation in some poeciliid lineages. There is some evidence that female mosquitofish may have such biases. For example, Gould et al. (1999) presented female *G. holbrooki* with male models modified with black patterns or enlarged fins. Females generally spent more time near the modified models than those that resembled wild-type males. However, when McCoy et al. (2011) presented female *Gambusia affinis* with animated male photographs digitally modified with rostral filaments (resembling those of male Mexican mollies, *Poecilia sphenops*), females spent more time near animations of unmodified males.

These results provide mixed insight into the possibility of sensory biases in female mosquitofish, but it

remains unclear whether the females in these studies recognized the models and images as conspecific males. We therefore conducted a study in which we experimentally applied ornamentation to living stimulus males to test whether colorful signals influence the behavior of female Western mosquitofish, *G. affinis*. A female preference for artificially colorful males would support the hypothesis that female sensory biases evolved early in the Poeciliidae.

Methods

The study followed a protocol approved by the Montclair State University Institutional Animal Care and Use Committee. In April 2011, we obtained living specimens of *G. affinis* from the Charles O. Hayford State Fish Hatchery in Hackettstown NJ and transported them to the laboratory in 40-l plastic coolers containing oxygenated aquarium water. Western mosquitofish are fairly easy to maintain in captivity (Pyke 2005). For 2 wk prior to experimentation, we housed mixed-sex groups of approximately 50 individuals in 35 × 15 cm aquaria at 21°C under a long day (16L:8D) photoperiod and provided commercial tropical fish flake food *ad libitum* each day. After the study ended, subjects were returned to a laboratory breeding colony and maintained thereafter.

We conducted four independent experiments to investigate whether female *G. affinis* exhibit affinity for stimuli with colorful ornaments. In each experiment, we observed the positional behavior of focal females in a glass observation aquarium (15 × 90 cm, Fig. 1) divided into left, center, and right sectors (30 cm each) with each sector labeled outside the visual field of fish inside the aquarium. Each focal female could freely swim between the sectors of the observation tank. We placed smaller aquaria (7.5 × 15 cm) immediately against the left and right

ends of the observation tank and inserted one-way mirrors between the glass walls of each tank. This permitted the focal female in the observation tank to view the contents of the adjacent tanks but prevented any fish in the adjacent tanks from viewing the focal female. Following the methods of Bisazza et al. (2001), we placed an additional tank (7.5 × 15 cm) containing three companion females immediately behind the middle virtual sector of the observation tank (Fig. 1). When exposed to harassing males, groups as small as two female *Gambusia* tend to shoal together (Dadda et al. 2008), so proximity of companion females in the central sector presumably created a social refuge for the focal female. We strategically placed illumination to ensure that the focal female could view the three adjacent tanks, but would be unlikely to detect two human observers positioned two meters away on the fourth (open) side of the observation tank (Fig. 1). Stimulus fish in the flanking aquaria also did not noticeably react to the presence of human observers, nor did they show evidence of interacting with their reflections in the one-way mirrors.

For all behavioral trials in this study, we randomly selected a new focal female and three new companion females from different mixed-sex holding tanks. We provided each individual a 30-min acclimation period in the experimental tanks prior to data collection (previous studies have reported acclimation periods from 10 min (Bisazza & Pilastro 1997; Dadda et al. 2008) to 60 min (Dadda et al. 2008)). During the acclimation period, we placed opaque dividers between the observation tank and the left and right adjacent stimulus tanks (Fig. 1). As each trial began, we removed the dividers and recorded the total amount of time each focal female spent in the left, center, and right virtual sectors during a 30-min focal observation period using *Noldus Observer* (2.0) event recording software. We
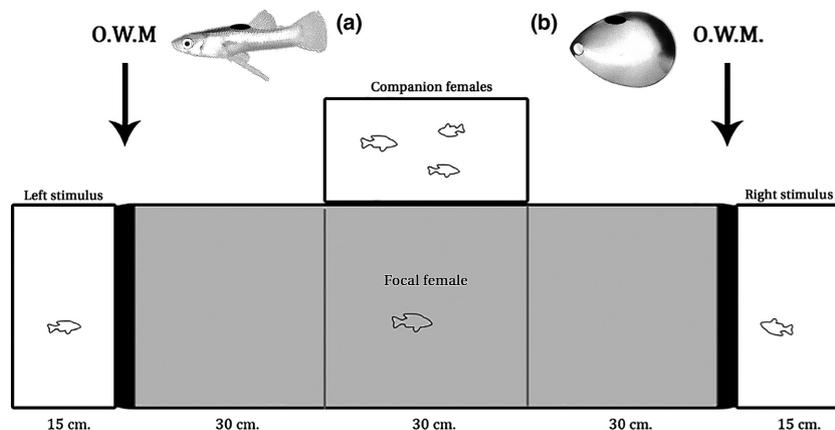
Fig. 1: Apparatus used for testing female positional affinity to different stimuli. One-way mirrors (O.W.M.) permitted the focal female to view the contents of the left and right tanks but prevented the occupants of the adjacent tanks from viewing the focal female. The lines dividing the central tank into three virtual sectors are for illustration only. Living stimulus fish (inset a) were treated with an ornament applied anterior to the dorsal fin as indicated by the black ellipse. Models (inset b) were prepared with an ornament applied as indicated by the black ellipse.

conducted experiments between 1200 and 1700 h, a range in which we frequently observed male sexual behavior in the laboratory setting. Although observers were not blind to experimental treatments, the same individuals recorded all observations and agreed on all recorded events.

Experiment One

In Experiment One (MALE-BLUE), we tested whether focal females show a positional affinity for males artificially ornamented with blue pigmentation. We selected an experimental hue similar (to the human eye) to the blue spots of male guppies, *Poecilia reticulata* (Kodric-Brown 1985). Because female mosquitofish are known to associate with larger males (Hughes 1985; Bisazza 1993), we randomly selected two males of approximately matched body size (no stimulus pair in any experiment differed by more than 6% in head to tail length) from different mixed-sex holding tanks and randomly assigned one to the left adjacent tank and one to the right adjacent tank.

While transferring males between tanks, we briefly (~ 30 s) and gently restrained each male in a sponge cutaway saturated with aquarium water. Before placing a male in an adjacent tank, we measured head to tail length and administered a small streak of blue (pigmented) or clear (unpigmented) fingernail polish (Forsythe Cosmetic Group, Ltd.; blue AN14 or clear top coat sealer) immediately anterior to the dorsal fin. We chose AN14 polish because it resembles (to the human eye) 420-nm light, which Endler et al. (2001) used to demonstrate blue spectral sensitivity in guppies (see Discussion). Although these pigments use acetate solvent, we chose them because preliminary trials with subcutaneous dyes (typically used in fish mark/recapture studies) produced dull signals and water-based external dyes did not persist in the aquatic medium. Pigmented lacquer usually became detached within 2 h of application and did not appear to adversely affect the treated fish (e.g., when males were returned to holding tanks, we observed female pursuit and feeding behavior).

When conducting more than one trial on the same day, we used the same pair of stimulus males for subsequent experimental trials to minimize the number of stimulus animals required for the study. In these cases, we alternated the placement of the pigmented and unpigmented males in the left and right adjacent tanks (Fig. 1) to account for factors like laterality (Aronson & Clark 1952) or other uncontrolled environmental factors like magnetic or electric fields

(Chew & Brown 1989). Males acclimated in each stimulus tank for 30 min prior to data collection. For Experiment One, we conducted a total of 20 trials with n = 20 focal females, n = 26 stimulus males (pairs used more than once on 7 occasions), and n = 60 companion females.

Experiment Two

To determine whether any female preference with respect to pigmentation in Experiment One was specific to *males*, we conducted a second experiment (Experiment Two, FEMALE) in which we tested whether focal females show an affinity for artificially pigmented female *G. affinis*. We replicated the conditions of Experiment One, changing only that females, rather than males, were used as pigmented and unpigmented stimulus animals in the left and right adjacent tanks. We conducted a total of 20 trials with n = 20 focal females, n = 36 stimulus females (pairs used more than once on 2 occasions), and n = 60 companion females.

Experiment Three

To determine whether any female preference in the first two experiments was associated with *living* conspecific animals, we conducted a third experiment (Experiment Three, MODEL) in which we tested whether focal females show an affinity for artificially pigmented inanimate objects (Tinbergen 1951; Gould et al. 1999). Tinbergen (1951) found that male three-spined stickleback, *Gasterosteus aculeatus*, attacked inanimate silver objects resembling the color, but not necessarily the shape, of male conspecifics. Following this approach, we tested whether female *G. affinis* respond to dorsally blue objects that do not closely resemble the shape of a mosquitofish. We replicated the conditions of Experiment One, changing only that silver spinner fishing lures (Cabela's: 1.5" length, Product # IK-310027), rather than living fish, were used as pigmented and unpigmented stimuli in the left and right adjacent tanks. These objects (3.8 cm) have a silver reflective surface and a lachrymiform shape with a small hole inside the tapered end, but are otherwise unadorned. Oriented with the tapered end sideways, we applied polish to the dorsal rim of the models in the same manner we applied it to live stimulus fish in previous experiments (Fig. 1). We alternated the placement of the pigmented and unpigmented models in the left and right adjacent tanks with each subsequent trial for a total of 19 trials with n = 19 focal females and n = 57 companion females. The same

pigmented and unpigmented stimulus models were used in all trials.

Experiment Four

To determine whether any female preference in Experiment One was associated the color of the male ornament, we conducted a fourth experiment (Experiment Four, MALE-RED) identical to Experiment One, but used red nail polish (Forsythe Cosmetic Group, Ltd; Red 115) instead of blue polish. We chose Red 115 polish because it resembles (to the human eye) 660 nm light, which Endler et al. (2001) used to demonstrate red sensitivity in guppies (see Discussion). We conducted a total of 20 trials with n = 20 focal females, n = 34 stimulus males (pairs were used more than once on 3 occasions), and n = 60 companion females.

Chromatic Measurement of Ornaments

To measure hue, saturation, and brightness value (HSV) of the blue and red ornaments, we applied streaks of both pigments to the same white sheet of photocopy paper and photographed them together under room lighting and a camera flash. We then analyzed the image with an online Image Color Summarizer tool available at http://mkweb.bcgsc.ca/color-summarizer. This tool calculates average hues of similar pixel clusters using mean of circular quantities. The tool identified the blue pigment as matching HEX #1C2879, with hue(H) = 232, saturation(S) = 77, and brightness value(V) = 47. The red pigment matched HEX#751215, with hue(H) = 358, saturation(S) = 85, and brightness value(V) = 46. Saturation and brightness of the pigments were relatively similar, and we anticipated that any differences in behavior would indicate responses to hue.

Statistical Analysis

Results were analyzed with JMP® Pro (11.0) statistical software with α = 0.05. We did not assume that data were normally distributed and used nonparametric statistical procedures to analyze the untransformed data. For each experiment, we used the Wilcoxon matched-pairs signed-rank test to compare the amount of time focal females spent on each side of the observation tank and the Spearman's rank correlation coefficient to examine the relationship between body size of stimulus fish and the amount of time focal females spent near them. We used the Wilcoxon test to compare the total amount of time focal females

spent near male conspecifics (combined data from experiments One and Four) vs. the total amount of time focal females spent near stimuli that were not male conspecifics (combined data from experiments Two and Three). We also used the Kruskal–Wallis test to compare stimulus size, time spent on left vs. right sides, and time spent near pigmented vs. unpigmented stimuli across the four experimental groups. In the event of significant effects between experimental groups, *post hoc* pairwise comparisons of experimental groups were made using the Wilcoxon method for nonparametric comparisons.

Results

Stimulus Size

There were no significant differences between the lengths of pigmented and unpigmented stimuli in any treatment group (Wilcoxon test, MALE-BLUE, S = −1.5, p = 0.9635; MALE-RED, S = 6, p = 0.8210; FEMALE, S = −31, p = 0.2563; MODEL, S = 0, p = 1.0000). There were no significant effects when differences in length between the two stimuli were compared across the four experimental groups (Kruskal–Wallis, p = 0.6291).

Left vs. Right Sides

There were no significant differences between times spent on the left vs. right side of the focal tank in any experimental treatment (Wilcoxon test, MALE-BLUE, S = −41, p = 0.1327; MALE-RED, S = 9.5, p = 0.7019; FEMALE, S = 29, p = 0.2943; MODEL, S = −25, p = 0.3321). There were no significant effects when differences in the time spent on left and right sides of the observation tank were compared across the four experimental groups (Kruskal–Wallis, p = 0.3609).

Pigmented vs. Unpigmented Stimuli

When focal females were presented with blue-pigmented vs. unpigmented male fish, they spent significantly more time on whichever side of the tank the blue-pigmented male was located (Fig. 2, Wilcoxon test, MALE-BLUE, S = −71, p = 0.0064). In seven cases, the same stimulus males were presented to more than one female. We therefore removed the subsequent female in each case from analysis to account for possible non-independence among females that were exposed to the same males. The results of this more conservative analysis were similar:
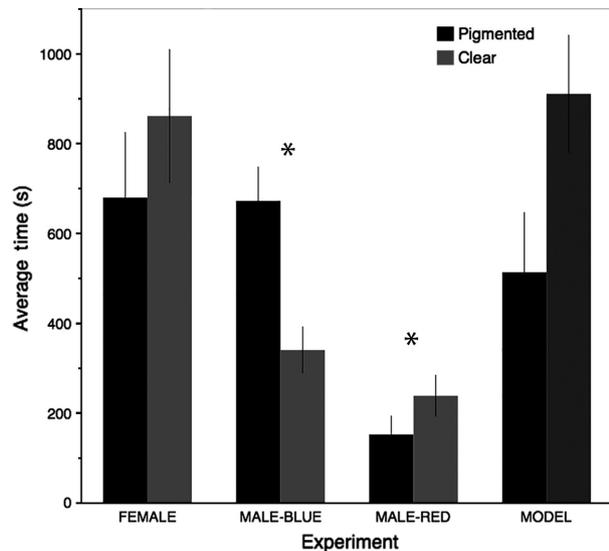
Fig. 2: Mean time (s) focal females spent near pigmented stimuli (dark bars) and clear-coated stimuli (gray bars). Asterisks indicate significant differences between matched pairs. Error bars represent one standard error on the mean.

Females spent significantly more time on whichever side of the tank the blue-pigmented male was located (Wilcoxon test, S = −28.5, p = 0.0479). There was, however, no relationship between time spent near a male and size of the male (blue males, Spearman's ρ = −0.0604, p = 0.8445; clear males, Spearman's ρ = −0.1319, p = 0.6676).

Focal females exhibited the opposite effect, however, when presented with red-pigmented males, spending significantly more time on whichever side of the tank the unpigmented male was located (Fig. 2, Wilcoxon test, MALE-RED, S = 68.5, p = 0.0016). In three cases, the same stimulus males were presented to more than one female. We therefore removed the subsequent female in each case from analysis to account for possible non-independence among females that were exposed to the same males. The results in this more conservative analysis were similar: Females spent significantly more time on whichever side of the tank the unpigmented male was located (Wilcoxon test, S = 59.5, p = 0.0032). There was, however, no relationship between time spent near a male and size of the male (red males, Spearman's ρ = −0.2495, p = 0.3341; clear males, Spearman's ρ = −0.0666, p = 0.7994).

When focal females were presented with blue-pigmented vs. unpigmented female fish, there were no significant differences in time spent on either side of the tank (Fig. 2, Wilcoxon test, FEMALE, S = 22,

p = 0.4304). In two cases, the same stimulus females were presented to more than one focal female. We therefore removed the subsequent female in each case from analysis to account for possible non-independence among focal females that were exposed to the same stimulus females. The results were similar: There were no significant differences in time spent on either side of the tank (Wilcoxon test, S = 14.5, p = 0.5509). There was also no relationship between time spent near a stimulus female and size of the stimulus female (blue females, Spearman's ρ = −0.0709, p = 0.7797; clear females, Spearman's ρ = −0.0155, p = 0.9513). There were also no significant differences in time spent on either side when focal females were presented with blue-pigmented vs. unpigmented spinner lures (Fig. 2, Wilcoxon test, MODEL, S = 37, p = 0.1447).

Focal females spent significantly less time in the left and right tank sections when the stimuli were males (regardless of color), spending more total time in the central section in proximity to the companion females (Fig. 2, Wilcoxon test, S = 2167, p < 0.0001). There were significant effects when differences in the time spent near pigmented and unpigmented stimuli were compared across the four experimental groups (Kruskal–Wallis, p = 0.0117). *Post hoc* pairwise comparisons indicated that the differences between time spent near pigmented vs. unpigmented stimuli were significantly greater in the experiment with blue males than the experiment with red males (p = 0.0006), and also greater in both experiments with stimulus males than the experiment with models (blue males, p = 0.0086 and red males, p = 0.0410, respectively).

Discussion

Focal females in this study exhibited a positional bias when male conspecifics displayed a colorful ornament. This did not appear to be a general response to colorful signals, however, because ornamented females and models did not elicit a significant response. That females only responded to *males* suggests that the behavior had a sexual context. Females were attracted to males with blue ornaments, with opposite results for red ornamentation. This suggests that red coloration had a repulsive effect. For example, if red color is normally associated with bleeding, females might avoid males that appear unhealthy or injured. It is also possible that females merely found males with clear polish more attractive than those with red ornaments. Clearly females were able to

detect and discriminate between the two pigments, but why did they prefer blue?

One possibility is that the blue pigment caused a male to appear darker and more melanistic. Notwithstanding evidence that female mosquitofish generally do not prefer melanistic stimulus males (Martin 1986; Nelson & Planes 1993; Bisazza et al. 2001), we find this possibility unlikely. The blue pigment (to the human eye at least) contrasted with the darker scales to which it was applied. Perhaps the blue pigment caused males to appear less melanistic and therefore more attractive to females. However, this would not explain the opposite response to red ornaments, which also contrasted with the darker background. Because both pigments had similar brightness and saturation values, the more likely possibility is that females responded to differences in hue.

Could responsiveness to blue and red ornaments in female mosquitofish result from shared sensitivity to these wavelengths in the Poeciliidae? Endler (1980) found that spots with blue structural color in male guppies increased rapidly under intersexual selection following predator exclusion. Female guppies also prefer male ornaments with red, orange, and yellow carotenoid pigments (Kodric-Brown 1985; Houde 1987). Endler et al. (2001) demonstrated that variation in sensitivity to blue (420 nm) vs. red (660 nm) wavelengths in guppy populations is heritable and that spectral sensitivity evolves in response to artificial selection for red vs. blue sensitivity. The proximate mechanism for guppy spectral sensitivity (Archer & Lythgoe 1990) appears to be retinal cones with peak absorbance at 408 nm (blue sensitivity) and cone classes with peak absorbance between 533 and 572 nm (red sensitivity). These cells are likely homologous in the Poeciliidae: Rod opsin sequences in *P. reticulata* and *G. affinis* are 98.6% identical (Archer & Hirano 1997). In the present study, female responses to red and blue ornaments suggest that spectral sensitivity could evolve in populations of *G. affinis* if there is corresponding heritable variation in these homologous opsins.

Consistent with the results of the present study, others have demonstrated chromatic discrimination in mosquitofish. Russo et al. (2008) found that *G. affinis* strike at green fishing lures more often than at yellow or red lures. Female *G. affinis* also shoaled with yellow animated female images more than red animated images (Polverino et al. 2013). Although these studies do not report hue, saturation, and brightness of color cues, the results suggest that mosquitofish avoid (as in the present study), or

at least do not respond to, red coloration. Male mosquitofish exhibit color discrimination as well, specifically for the yellow spot near the female urogenital opening. Kodama et al. (2008) demonstrated that males attempted to copulate more often with yellow-spotted female models than those with black, gray, or white coloration. These results contrast with those of Russo et al. (2008), indicating that responsiveness to yellow may differ in mating and feeding contexts.

Color discrimination behavior and homologous retinal opsins in *Gambusia* and *Poecilia* suggest a common poeciliid ancestor with these characteristics. Did ancestral poeciliid males have corresponding chromatic signals? There are two general possibilities: First, ancestral males may have had colorful ornaments that were subsequently lost in most lineages (which may be the case in *Xiphophorus sp.*, see Meyer et al. 1994). Alternatively, ancestral species may have lacked sexual dichromatism and male color subsequently evolved in only some lineages. This is apparent in the anuran genus *Physalaemus*, for which behavioral and phylogenetic studies suggest an ancestral female auditory bias that drove evolution of complex male courtship signals in some, but not all, descendant species (Ryan & Rand 1993).

As discussed by Bisazza et al. (1997), the poeciliids are closely related to the cyprinodontiformes (killifishes), in which it is typical for males to display bright coloration (Foster 1967). Perhaps early poeciliid males shared this characteristic. However, the authors concluded that the alternative hypothesis was more likely, citing two patterns of evidence. First, the majority of poeciliid species are characterized by gonopodial thrusting without courtship (Bisazza 1993). Second, the most primitive extant poeciliid, *Tomeurus gracilis* (Meyer & Lydeard 1993), exhibits gonopodial thrusting but not male ornamentation (Gordon 1955).

If the weight of evidence points to a preexisting sensitivity for red and blue wavelengths, it seems surprising that colorful male ornaments are not more common in the Poeciliidae. The results of the present study suggest that male *G. affinis* with blue ornaments would have a mating advantage if there were corresponding heritable variation for coloration. Indeed, the evolution of mating coloration is not unknown in the genus: Yellow and orange pigments evolved in the dorsal and anal fins of male Bahamas mosquitofish, *Gambusia hubbsi.* Males display these signals during courtship, and female *G. hubbsi* have been shown to prefer male models with more orange dorsal fin

ornamentation (Heinen-Kay et al. 2015). Much like guppies (Endler 1980), male colors in *G. hubbsi* are more pronounced in populations without predators (Martin et al. 2014).

Female responses to colorful male ornaments in *G. hubbsi* and *G. affinis* indicate that female preferences are shared in the genus even though male ornaments are not. Perhaps the differences in male ornamentation and courtship behavior among species are associated with environmental factors (Farr 1989; Bisazza 1993). Many poeciliids occupy diverse or fluctuating environments, and there is some evidence that poeciliid species with courtship tend to be specialists living in predictable habitats (Farr 1984). Even in poeciliid species with male ornaments, populations in low-visibility conditions are characterized by proportionately higher gonopodial thrusting and lower levels of courtship behavior (Endler 1987).

Ultimately, the question of rare ornamentation in the Poeciliidae may be difficult to answer because male signals that correspond to female sensory biases can be evolutionarily lost only to subsequently reappear. This appears to be the case in *Xiphophorus*, as a DNA-based phylogenetic revision to the genus suggests that sword-shaped tails are in fact the ancestral character state (Meyer et al. 1994). This conclusion is consistent with earlier studies that used hormone treatments to induce development of elongated ventral caudal fin rays of *X. maculatus*, a species that does not normally develop a sword (Gordon et al. 1943). Swordless *X. maculatus* are speculated to have had ancestors with swords, as the code for sword development still resides in the genome.

Whether the ancestors of mosquitofish were sexually dichromatic remains to be determined. Evolution of male ornaments in *G. affinis* is contingent upon several factors, such as the chance occurrence of mutations that generate heritable male signal variation that corresponds to female preferences (Qvarnström et al. 2006). Male ornaments, should they appear, must also confer mating advantages that exceed the potential costs imposed by harmful 'eavesdroppers' like predators and parasites (Zuk & Kolluru 1998; Page & Ryan 2008). Also, the prevalence of gonopodial thrusting in the Poeciliidae indicates that coercive mating confers high fitness, and female preference for male ornaments may not provide a reproductive advantage to males in many poeciliid species (Farr 1989; Bisazza 1993). Perhaps future developmental and phylogenetic studies will shed light on the evolutionary interplay between female sensory systems and male mating strategies in *Gambusia*.

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