

# Do I Know You? Species Recognition Operates Within and Between the Sexes in a Unisexual–Bisexual Species Complex of Mollies

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## Abstract

Male sailfin mollies (*Poecilia latipinna*) can be sexually parasitized by closely related, unisexual, gynogenetic Amazon mollies (*Poecilia formosa*). This study examined possible cues used by male *P. latipinna* to distinguish between conspecific females and sympatric, heterospecific *P. formosa*. Digital photos were used to create models to test male *P. latipinna* preference for model female *P. latipinna* and *P. formosa* with a full suite of traits and altered models of *P. latipinna* and *P. formosa*. Male *P. latipinna* significantly preferred models of either species over no stimulus, demonstrating that models elicit a male response. Males also significantly preferred female *P. latipinna* models over *P. formosa* models. We also examined species recognition by female sailfin mollies using the same models, and found that female sailfin mollies significantly preferred to associate with female *P. latipinna* over *P. formosa*. These results taken together suggest that the use of fish models yield results similar to those studies using live stimuli. Male preference was then tested for unaltered vs. altered models in the following combinations: (i) *P. formosa* vs. *P. formosa* with a female *P. latipinna* fin; (ii) *P. formosa* vs. *P. formosa* with a female *P. latipinna* lateral spot pattern; (iii) *P. formosa* vs. *P. latipinna* with a *P. formosa* fin and their spotless lateral pattern. Males did not significantly prefer models with any isolated traits over the unaltered *P. formosa* models. Thus, males may be using traits other than the ones isolated for species recognition or males may be using a suite of multiple traits to recognize conspecific females.

## Introduction

When choosing a mate in a species where closely related heterospecifics are sympatric, individuals are expected to choose a genetically compatible conspecific and avoid heterospecifics (Dobzhansky 1937). To do this, animals may assess multiple morphological and behavioral traits, as well as weigh the relative importance of each trait. Candolin (2003) reviews the current hypotheses to explain the evolution of multiple cues including the back-up signal

hypothesis, the multiple message hypothesis, and the species recognition hypothesis. The backup signal hypothesis focuses on multiple cues providing information about a single quality to increase accuracy of assessment of that quality (Johnstone 1996). The multiple message hypothesis, in contrast, states that each multiple signals provide information about a different aspect of quality (Johnstone 1996). A specific extension of the multiple message hypothesis, the species recognition hypothesis, proposes that cues used to identify a genetically compatible mate will

differ from those used to identify a high-quality mate (Pfennig 1998, 2000).

Several studies have examined the use of multiple cues as a mechanism of species recognition. For the gray tree frog (*Hyla chrysoscelis*), different cues have individual importance. Female gray tree frogs assess pulse rate of male calls for species recognition, whereas call duration indicates male genetic quality and is used for mate-quality recognition (Gerhardt 2001). In the swordtail *Xiphophorus pygmaeus*, females use cues detected by multiple sensory modalities: females use both a visual cue (vertical bars) as well as chemical cues to assess species identity (Hankison & Morris 2003). These females also prefer larger conspecific males (Hankison & Morris 2002), which could lead to mating with larger sympatric heterospecific males (*Xiphophorus cortezi*) that overlap in size with *X. pygmaeus*. By testing female *X. pygmaeus* preference for vertical bars and chemical cues individually and in combination, Hankison & Morris (2003) found that both chemical cues and vertical bars were required for females to discriminate between relatively smaller conspecifics and larger heterospecifics. Thus, by using multiple cues, females relied on a 'backup' signal to avoid compromising between species and mate-quality recognition or mating with heterospecifics. The benefits of discriminating against a heterospecific and mating with a lower quality conspecific may be strong enough to balance the cost of assessing multiple cues (Pfennig 2000).

Individuals may not only evaluate multiple traits but also evaluate multiple components of a single composite trait. For example, female green swordtails (*X. helleri*) prefer males with swords, extensions of the ventral caudal fin rays that have dorsal and ventral black stripes and yellow/green coloration between the two black stripes. Using digital video technology, Basolo & Trainor (2002) isolated each component of the male sword and found that females significantly preferred swords with black components to those without any black. Females also exhibited a preference for the yellow coloration in the absence of black stripes, indicating that multiple sword components were evaluated by females.

It is especially relevant to determine the cues used for mate choice in species that risk mating with heterospecifics. One such example occurs in sympatric populations of *Poecilia latipinna* (Lesueur) and *Poecilia formosa* (Girard) where *P. formosa*, a unisexual gynogenetic species, must mate with males of closely related species (*P. latipinna* or *Poecilia mexicana*) to initiate embryogenesis (Hubbs & Hubbs 1946; Kall-

man 1962; Darnell et al. 1967). However, inheritance in the resulting offspring is strictly maternal (Hubbs & Hubbs 1932; Balsano et al. 1989). *Poecilia formosa* is a result of specific hybrid events between *P. latipinna* and *P. mexicana* and has persisted in natural environments for at least 100 000 yr (Avisé et al. 1991; Scharl et al. 1995; Dries 2003).

Male *P. latipinna* prefer to mate with conspecific females (Gabor & Ryan 2001), but mate with *P. formosa* as well. Four non-mutually exclusive hypotheses may explain why males continue to mate with heterospecifics: (i) female *P. latipinna* copy the mate choice of *P. formosa*, providing benefits for male *P. latipinna* that mate with *P. formosa* (Schlupp et al. 1994); (ii) *P. formosa* are more aggressive than female *P. latipinna* when interacting with male *P. latipinna* and thus elicit matings (Foran & Ryan 1994); (iii) *Poecilia formosa* share genes with *P. latipinna* and *P. mexicana* and may be falsely recognized as conspecifics by males of these species (Dries 2003); (iv) there is a conflict in species and mate-quality cues used by male *P. latipinna* when selecting mates that may be taken advantage of by *P. formosa*. Gumm & Gabor (2005) found that in four out of five sympatric populations surveyed, males presented with a choice of mating either with relatively smaller conspecific females or relatively larger *P. formosa* do not prefer conspecifics. Larger size indicates greater fecundity in *P. latipinna* (Travis et al. 1990; Trexler et al. 1997) and therefore, these results suggest a conflict between mate-quality and species recognition cues that leads to males mating with Amazon mollies. At present, the cues that are important for species recognition by male *P. latipinna* are unknown, and it is possible that both visual and chemical cues are important. Visual cues are sufficient, as Schlupp et al. (1991) showed that male *P. latipinna* can distinguish between female *P. latipinna* and *P. formosa* with visual cues alone and they significantly preferred to associate with *P. latipinna*.

The hybrid origin of *P. formosa* has resulted in an intermediate morphology between the parental species (Hubbs & Hubbs 1946; Dries 2003). *Poecilia formosa* has fewer dorsal fin rays (10–12) than *P. latipinna* (13–15), and the dorsal fin is positioned more posterior in female *P. formosa* than in *P. latipinna* (Hubbs & Hubbs 1932). There is also a lateral spot pattern present on the body of *P. latipinna* that *P. formosa* lack (Lee et al. 1980). To determine which visual traits males may be using for species recognition, this study examined if these differences affect association preferences in male *P. latipinna*. Although association preferences may not always be due to

mating preferences *per se* (Gabor 1999), association times reflect general social preferences that could lead to differential mating choices.

The first objective of this study was to use model fish to examine the ability of both male and female sailfin mollies to differentially associate with conspecific females over heterospecific females. The second objective was to then examine the relative importance of three individual visual cues (fin placement, spot pattern, and body shape) that may be used by male *P. latipinna* for species recognition. Models provide the possibility of precise manipulation of traits and allow avoidance of technical limitations set by using live stimuli. Examining individual cues may lead to greater understanding of species recognition under more complex scenarios, such as when cues conflict. Models used in the present study were formulated after *P. latipinna* models used by MacLaren et al. (2004). We approached our objectives by testing: (i) male preference for unaltered models of *P. latipinna* and *P. formosa* vs. no stimulus; (ii) male preference for unaltered female *P. latipinna* models vs. unaltered *P. formosa* models; (iii) female preference for unaltered female *P. latipinna* models vs. unaltered *P. formosa* models; and (iv) male preference for models that isolate traits that differ between female *P. latipinna* and *P. formosa*.

## Methods

### Collection and Maintenance

The population of *P. latipinna* used in this study was collected during Mar. 2003 from the Tamaulipas region of Mexico [latitude, longitude (UTM) 25.07, 98.02] and is naturally sympatric with *P. formosa*. Both *P. latipinna* and *P. formosa* were collected by seine and dip-net and were transported to Texas State University (San Marcos, TX). The fishes were maintained in a laboratory in 38-l aquaria (54 × 29 × 33 cm) at a constant temperature (22–25°C) and were maintained on a 14.00:10.00 hours light:dark cycle with UV fluorescent lighting that simulates daylight (40 W Coralife Day-Max Aquarium daylight, 40 W Coralife Actinic 03 Blue, 40 W Coralife 10 000 k high intensity purified super daylight, and 40 W regular fluorescent (General Electrics, Cleveland, OH, USA)). Fishes were fed spirulina and freshwater flake food (Ocean Star International Inc., Marine Laboratory, Hayward, CA, USA) twice daily, supplemented with live and freeze-dried brine shrimp. Only mature males, identified by the fusion of the anal fin into the gonopodium, were used in

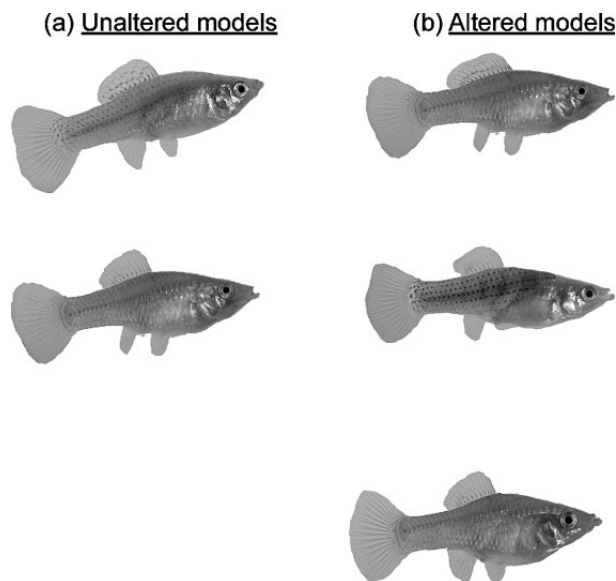
trials. Mature female *P. latipinna* and *P. formosa* that served as 'subject females' originated from the same population as the males that we tested. When choosing 'subject females', we avoided females that were visibly gravid so that females would be in similar stages of the brood cycle.

### Isolation of Species-Specific Cues

Digital photos were taken of 18 females per species from the sympatric population. For photos, each individual was isolated in a small section (8 × 4 × 6 cm) of a 19-l aquarium (40 × 20.5 × 26.5 cm) with Plexiglas dividers. The aquarium was filled with 20 cm of water with white paper covering the back to provide a background for the photos. The aquarium was in a dark room, which excluded outside light and facilitated consistent lighting for all of the pictures. A 15-W Sun-Glo full spectrum light (General Electric, Cleveland, OH, USA) was placed directly above the aquarium and a 60-W Plant Gro and Show light (General Electric) was placed in front of the aquarium to enhance definition of the images. Pictures were taken with a digital camera (Nikon Digital CoolPix 950, Nikon Inc., Melville, NY, USA) placed 15 cm from the aquarium. The setup of the tank allowed pictures to be taken when fishes had all fins extended. In addition, all fishes were at a similar distance and angle to the camera.

To prepare the photos for construction of models, Adobe Photoshop 5.5 (Adobe Systems Inc., San Jose, CA, USA) was used to cut out each image. To control for size differences, each image was then 'free transformed' to 38 mm, the  $\bar{x}$  size of females (*P. latipinna* and *P. formosa*) for the population ( $\bar{x} \pm SE = 38.0 \pm 0.37$  mm; range from 30.1 to 45.4 mm). Although fishes may differ in characteristics on each lateral side, only one side was used to minimize this effect on male preference. The side used for each image was haphazardly selected based on the highest quality image available for each fish. Each fish image and its horizontally flipped image were then printed onto transparencies using a Hewlett Packard 7350 printer (Hewlett Packard, Palo Alto, CA, USA). Mirror images were cut out and glued together with a piece of white paper cut in the shape of the fish's body (excluding the fins) between the two transparencies. The resulting two-dimensional models have detailed opaque bodies and transparent fins (Fig. 1).

To isolate individual variables, the 18 *P. latipinna* images were randomly paired with a *P. formosa* image. Pictures were digitally manipulated to make altered models from these paired images. These pairs



**Fig. 1:** (a) Unaltered model female *Poecilia latipinna* (top) and *Poecilia formosa* (bottom). (b) Hybrid models of *P. formosa* with *P. latipinna* fin (top); *P. formosa* with *P. latipinna* spot pattern (middle); and *P. formosa* fin and lack of spot pattern (bottom)

were used to create all three altered models. For example, a *P. formosa* was paired with a *P. latipinna*, and to create an altered model that isolated the dorsal fin differences, we cut the dorsal fin of the *P. formosa* from its picture and replaced it with the dorsal fin from the *P. latipinna*. The fin was rotated to best fit the natural slope of the dorsal surface and positioned the same distance from the snout as it had been on the *P. latipinna* image using a background grid for measurements. To create an altered model that isolated the lateral spot pattern, the images of both fish were aligned on a grid and Adobe Photoshop 5.5's 'rubber stamp' tool (a cloning brush that samples from a set point on an image and paints to another) was used to transfer the *P. latipinna* lateral spot pattern directly onto the *P. formosa* body in the exact same area. Finally, to control for potential shape differences between the species, the dorsal fin and spotless lateral body pattern of the *P. formosa* were transferred in the same manner onto the *P. latipinna* image. The resulting hybrid models ( $n = 18$ ) consisted of: (i) a *P. formosa* with a *P. latipinna* fin; (ii) a *P. formosa* with *P. latipinna* lateral spot pattern; and (iii) a *P. latipinna* with *P. formosa* fin and lacking the lateral spot pattern (Fig. 1).

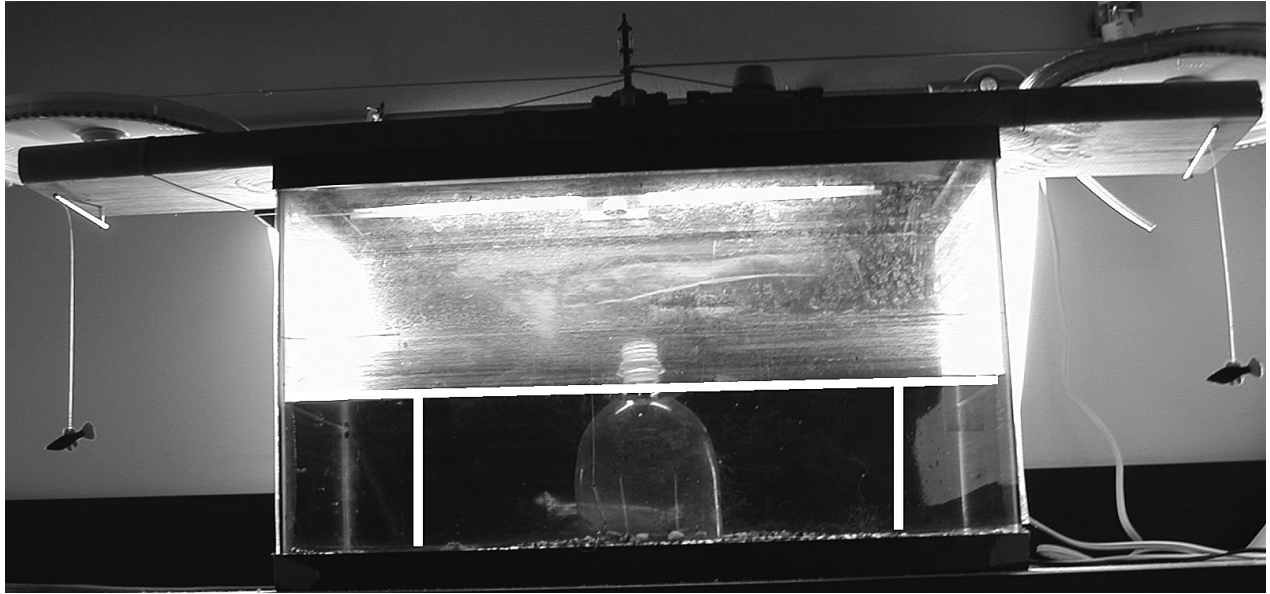
To animate the models, a motorized pulley system was created using a 120-V AC motor (General Electrics, Cleveland, OH, USA) that was taken from a fan. The motor was operated using a common household

dimmer switch, and attached to a wooden board ( $91.4 \times 15.2 \times 2.5$  cm) placed directly on top of the aquarium. Small rubber bands ran from the shaft of the motor (0.6-cm diameter) to a plastic spool (1.3-cm diameter) and elastic bands then ran to plastic disks (24.8-cm diameter) which served as the pulley. A metal bar (11.4 cm) was attached to the center of each pulley. Fishing line (16 cm) was tied to this L-shaped metal bar so that when models were glued to the fishing line, they rotated in a circle (22.9-cm diameter) clockwise on each side of the tank (Fig. 2). Therefore, the models passed the side of the tank at a distance of approx. 3 cm. The speed of the stimulus models was constant within each trial and was approximately 17 rotations per minute.

#### Time Trials

Trials conducted in a 38-l aquarium ( $54 \times 29 \times 33$  cm) that contained tan gravel and 24 cm of aerated and filtered water. A 15-W Sun-Glo full spectrum light (General Electric) was placed directly on top of the aquarium and two standard fluorescent lights were placed at an approx.  $45^\circ$  angle 5 cm from the back of each side of the aquarium. This illuminated both the model fish and the test fish. The aquarium was placed in a darkroom to block out all other light sources and the back-side was covered with black plastic to prevent test fishes from being distracted by the environment around the tank. The sidewalls of the testing chamber (35 cm from the sides of the tank) were lined with white poster board to provide a uniform background for the models. The front of the tank was covered with one-way film to minimize disturbance of the fishes by the observer. All fishes were fed prior to testing. Trials using males were conducted from Jul. to Oct. 2003 between 09:00 and 17:00 hours, and trials testing females were conducted from Jun. to Aug. 2004 between 09:00 and 17:00 hours.

Markings on the one-way film divided the aquarium into three sections: the two outer sections (9 cm of each end of the aquarium) were the choice sections while the inner section was the no choice area. After attaching the models to the pulley system and turning on the motor, a test *P. latipinna* was placed in the center of the aquarium under a clear plastic cylinder (12-cm diameter  $\times$  15 cm) and allowed to acclimate for 10 min. During this acclimation period the test subjects could see but not interact with the models. After release, time spent by the test fish in each of the choice sections of the tank was recorded for 10 min. After reversing the



**Fig. 2:** Experimental set up for testing male *Poecilia latipinna* association preference for model female fish. The white lines delineate the outer preference zones

sides of the models, the test fish was allowed 10 min to re-acclimate with the models rotating before running the trial again. This controlled for any potential side bias of the test fish. The initial left-right position of the transparencies was randomized between tests and each transparency was randomly selected from the 18 models of each species and/or hybrids. Although individual models were used more than once (i.e. there were 18 models of each species and hybrids, but up to 30 tests per expt), no two models were ever paired together more than once. Therefore, each pair of models was a novel set of stimuli.

#### Expt 1a: Model Control

To test male preference for unmanipulated models, we tested males ( $n = 30$ ) with (i) *P. latipinna* model vs. no stimulus; and (ii) *P. formosa* model vs. no stimulus. The order of the treatments was randomized, and males were tested at an interval of 24 h between treatments.

#### Expt 1b: Male *P. latipinna* Species Recognition

Following the model control trials, we tested the males from expt 1(a) for their ability to discriminate between the two species by testing the males ( $n = 30$ ) with a *P. latipinna* model vs. a *P. formosa* model.

#### Expt 2: Female *P. latipinna* Species Recognition

We assessed the ability of female *P. latipinna* to discriminate between the two species by testing females ( $n = 30$ ) with a *P. latipinna* female model vs. a *P. formosa* model.

#### Expt 3: Male *P. latipinna* Preference for Altered Models

Male preference for altered models was tested by randomly pairing a hybrid model with one of the unaltered *P. formosa* models. Hybrid models were paired with unaltered *P. formosa* because male *P. latipinna* would be expected to prefer unaltered *P. latipinna* models over any altered model, which does not have a full suite of *P. latipinna* characteristics. Alternatively, a male preference for any altered model with at least one *P. latipinna* trait might be expected over an unaltered *P. formosa* model. Therefore, by pairing the hybrid models with unaltered *P. formosa* models, males were expected to have a greater strength of preference for those hybrid models that have cues that they recognize as *P. latipinna* characteristics over the unaltered *P. formosa* models. Males [not previously tested in expts 1(a) and 1(b)] ( $n = 30$ ) were tested in the following treatments; (i) *P. formosa* vs. *P. formosa* with *P. latipinna* fin; (ii) *P. formosa* vs. *P. formosa* with *P. latipinna* spot pattern; and

(iii) *P. formosa* vs. *P. latipinna* with *P. formosa* fin and lack of spot pattern (Fig. 1b). All three treatments were randomized with an interval of 24 h between each treatment.

**Statistical Analyses**

The amount of time test individuals spent in the choice section on the right-hand side of the tank between trials within each treatment was compared using a paired t-test for all expts. We arbitrarily selected the data from the right side of the tank, as this avoids the possibility of lack of independence of data had the analysis been based on the total time spent with a given model stimulus, summed across trials within a treatment (after sides were reversed) (Gabor 1999). To determine which isolated trait from conspecific females elicited the greatest strength of preference in expt 3, the amount of time spent in the choice section on the right-hand side of the tank with unaltered *P. formosa* models was subtracted from the time spent in the choice section on the right-hand side of the tank with hybrid models, and this strength of preference was compared among the treatments using one-way ANOVA.

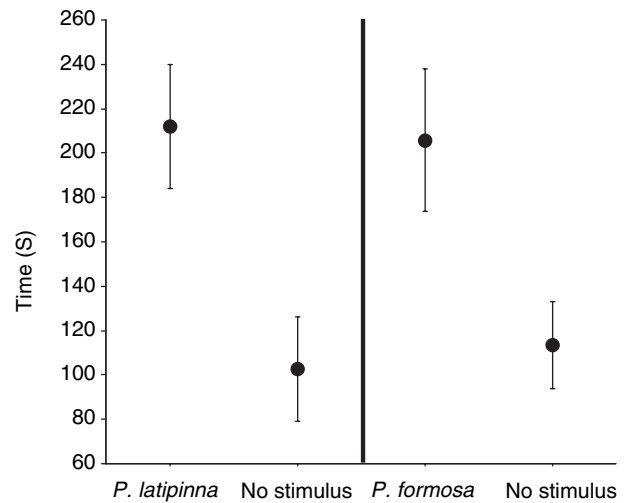
Test fish ‘responsiveness’ was also calculated for each trial as the total time spent in a ‘choice area’ per 1200 s (Gabor & Page 2003). Responsiveness scores near 1 indicate the subject either: (i) exhibited a strong preference for a particular model or (ii) exhibited a high degree of movement between the two stimuli, with little or no preference for either model. Conversely, fish with responsiveness scores near 0 indicate little interaction with either stimulus.

All variables were tested for assumptions of parametric tests, and were found to meet the assumptions. Furthermore, because individual males were tested on three subsequent days for expts 1(a), 1(b), and 3, we tested for any effect of order, irrespective of the treatment on time spent with subject females using a two-way ANOVA with model type and order as the main effects. All reported p-values are two tailed.

**Results**

**Expt 1(a)**

There was no significant effect of testing order on the time males spent associating with subjects (two-way ANOVA ‘order effect’:  $F_{(2,174)} = 0.550$ ,  $p = 0.5749$ ). Males significantly preferred to associate with *P. latipinna* models over no stimulus [paired t-test:  $t_{29} = 3.661$ ,  $p = 0.001$  (Fig. 3)]. Males also

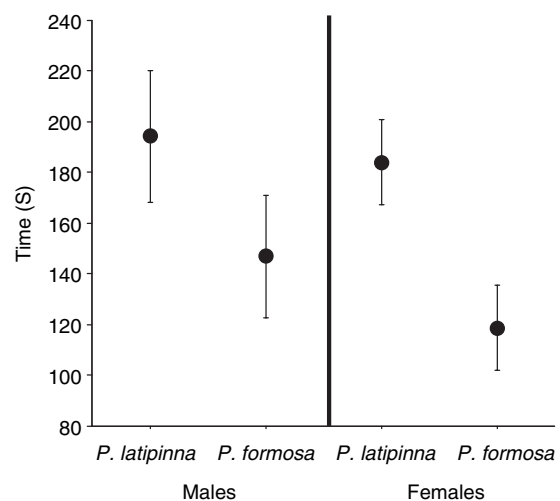


**Fig. 3:** Time spent by males with unaltered *Poecilia latipinna* models vs. no stimulus, and unaltered *Poecilia formosa* models vs. no stimulus

significantly preferred to associate with *P. formosa* models over no stimulus [paired t-test:  $t_{29} = 2.637$ ,  $p = 0.013$  (Fig. 3)].

**Expt 1(b)**

When testing for male species recognition of models, males significantly preferred *P. latipinna* models over *P. formosa* models [paired t-test:  $t_{29} = 2.761$ ,  $p = 0.0099$  (Fig. 4)]. Males showed similar levels of responsiveness for all treatments in expts 1(a) and 1(b) (Table 1), and responsiveness was not significantly different across the 3 d of testing (one-way ANOVA:  $F_{(2,87)} = 0.390$ ,  $p = 0.6781$ ).



**Fig. 4:** Time spent by males and females with unaltered *Poecilia latipinna* models vs. *Poecilia formosa* models

**Table 1:** Mean responsiveness and strength of preferences of male *Poecilia latipinna* toward unaltered and hybrid models

Expt	$\bar{x}$ responsiveness (s) $\pm$ SE	Strength of preference (s)
<i>P. latipinna</i> vs. no stimuli	0.488 $\pm$ 0.027	109.53 $\pm$ 29.51
<i>P. formosa</i> vs. no stimuli	0.473 $\pm$ 0.030	92.33 $\pm$ 33.12
<i>P. latipinna</i> vs. <i>P. formosa</i>	0.532 $\pm$ 0.033	47.37 $\pm$ 17.63
<i>P. formosa</i> vs. fin	0.580 $\pm$ 0.280	-14.33 $\pm$ 22.72
<i>P. formosa</i> vs. spot pattern	0.576 $\pm$ 0.034	-9.47 $\pm$ 27.26
<i>P. formosa</i> vs. shape	0.612 $\pm$ 0.025	1.70 $\pm$ 24.45

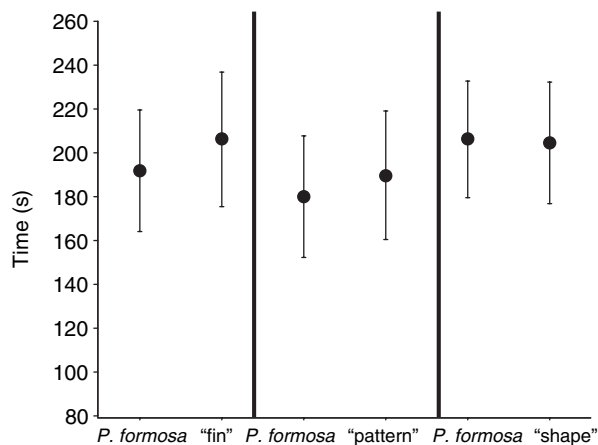
Responsiveness is measured as the total time spent(s) in a 'choice area' per 1200 s. Strength of preference is measured as amount of time spent (s) in the choice section on the right-hand side of the tank with the unaltered *P. latipinna* or *P. formosa* models minus the time spent (s) in the choice section on the right-hand side of the tank with either no stimulus or the hybrid models.

### Expt 2

When testing for female species recognition of models, females significantly preferred *P. latipinna* models over *P. formosa* models [paired t-test:  $t_{29} = 2.384$ ,  $p = 0.0239$  (Fig. 4)].

### Expt 3

There was no significant effect of testing order on the time males spent associating with subjects (two-way ANOVA 'order effect':  $F_{(2,174)} = 1.662$ ,  $p = 0.1928$ ), and there was no variation in association time based on model type (two-way ANOVA 'model effect':  $F_{(1,174)} = 0.103$ ,  $p = 0.7490$ ). Males had no significant preferences for any altered models over *P. formosa* models [(Fig. 5) paired t-tests: fin:  $t_{29} = 0.725$ ,  $p = 0.4745$ ; spot pattern:  $t_{29} = 0.247$ ,  $p =$

**Fig. 5:** Time spent by male *P. latipinna* with unaltered *P. formosa* models vs. hybrid models that isolate *P. latipinna* fin shape and placement, spot pattern and body shape

0.8064; shape:  $t_{29} = 0.032$ ,  $p = 0.9745$ ]. There was also no significant difference in strength of preference between the three types of altered models (one-way ANOVA:  $F_{(2,87)} = 0.176$ ,  $p = 0.8388$ ). Males showed similar levels of responsiveness for all treatments in expt 3 (Table 1), and responsiveness was not significantly different across the 3 d of testing the males in expt 3 (one-way ANOVA:  $F_{(2,87)} = 1.302$ ,  $p = 0.3606$ ).

### Discussion

When presented with unaltered models, male *P. latipinna* preferred to associate with female *P. latipinna* models over no stimulus. Male *P. latipinna* also preferred to associate with unaltered model *P. formosa* over no stimulus. These results demonstrate that models generate the expected response in male *P. latipinna* and that models are reasonable facsimiles of real fishes. Both males and females also preferred to associate with model female *P. latipinna* over model *P. formosa*. These preferences are not surprising given results of previous work showing that males prefer to associate with visual cues from live conspecific females (Schlupp et al. 1991), and that female *P. latipinna* can discriminate between live conspecific females and *P. formosa* (Schlupp & Ryan 1996). Our results with female sailfin mollies also provide further support for the hypothesis that heterospecific mate-choice copying by female *P. latipinna* is not a result of mistaken identity; females can discriminate between conspecific and heterospecific females (Schlupp & Ryan 1996).

Male *P. latipinna* did not significantly discriminate between unaltered *P. formosa* models and altered models that isolated the individual species-specific cues of dorsal fin size and placement, lateral spot pattern, and body shape. Further, there was no difference in strength of preference for any of the altered models. These results indicate that although the unaltered *P. latipinna* and *P. formosa* models resulted in significant male preferences, the data from this study do not support the hypothesis that the isolated visual cues of dorsal fin, spot pattern, and shape are sufficient as species recognition traits. Previously, Hubbs & Hubbs (1932) and Lee et al. (1980) demonstrated that spot pattern and fin size differ between female *P. latipinna* and *P. formosa*. We have found that female *P. latipinna* have dorsal fin to snout tip distances that are significantly less than *P. formosa* in the population used in our expts [*P. latipinna*  $\bar{x} \pm$  SE distance (mm) =  $18.51 \pm 0.18$ ; *P. formosa*  $\bar{x} \pm$  SE distance (mm) =  $21.03 \pm 0.32$ ; unpaired t-test:  $t =$

6.90,  $df = 54$ ,  $p < 0.0001$ ]. Currently, however, we do not know if there are meaningful differences in body shape. One way to test this possibility would be to carry out morphometric analyses for the two species for this population.

The low responsiveness scores in the trials testing unaltered models over no stimuli indicated that males spent more than half their time in the no choice compartment but still displayed a significant preference (Table 1). The responsiveness scores combined with high strength of preference for the unaltered *P. latipinna* over the unaltered *P. formosa* models suggest that in these trials, males were making a strong choice for the *P. latipinna* models (Table 1). In contrast, male responsiveness in the altered trials indicated that they spent more than half their time with stimuli, yet this was combined with low strength of preference (Table 1), suggesting that males in these trials were visiting both models but not significantly preferring either model.

If male *P. latipinna* assess multiple cues concurrently, individual cues would be insufficient to elicit a response. In this case, males might be expected to prefer the full suite of cues. Our results are congruent with these expectations. Combinations of cues can interact in a complex manner. To examine cue interactions, Kunzler & Bakker (2001) used computer-animated sticklebacks to assess the attractiveness of single and combined visual traits. Using virtual fish differing in red throat coloration, courtship intensity, body size, and combinations of these, Kunzler & Bakker (2001) found that male coloration increased female preference, whereas increased male courtship intensity did not. In addition, female stickleback preference increased as the number of traits available to judge male quality increased. Similar results are expected if male *P. latipinna* preference was tested for combinations of the cues that were isolated in the current study. However, it remains undetermined which cues in combination would be important factors in yielding strong male preferences.

An alternative mode of communication may also be important to male mating preferences. Males showed a significant preference for conspecific models over heterospecific models, indicating that visual cues alone are sufficient for species recognition by male *P. latipinna*. However, an alternative modality, such as chemical communication, may also be important to male mating preferences. Although chemical cues alone appear to be insufficient for males to recognize conspecifics (C. R. Gabor, unpublished data), it is possible that a combination

of visual and chemical cues may interact to provide the most reliable information to males about species recognition. In *X. pygmaeus*, both visual and chemical cues are important for species recognition. Hankison & Morris (2003) found an increase in variation when testing female *X. pygmaeus* preferences for males based on chemical cues followed by chemical and visual cues. Although not statistically significant, this difference in variation may be indicative of an interaction between the cues used for species recognition.

The lack of a population level preference for any individual cue may be related to the amount of variation shown by these males. Differences in preference between individual males may be consistent and negate each other, resulting in no population level preference (Wagner 1998). Thus, the high level of variation in male *P. latipinna* preference may indicate a polymorphism in male preference with some males consistently preferring the dorsal fin shape and placement, while others consistently prefer the lateral spot pattern or general body shape. By testing repeatability measures of female preference for male pigment patterns in *X. cortezi*, Morris et al. (2003) found that variation in preference was greater between females than within individuals. This indicates that there is a polymorphism in female preference with different females showing different preference extremes or no preference at all. Overall, no population level preference is seen by female *X. cortezi* for male pigment patterns. Repeatability of male preference was not tested in our expt, but it would be interesting to examine this given the conflict in species and mate-quality cues.

While males demonstrated no preference for what we perceive to be the obvious visual differences between female *P. latipinna* and *P. formosa*, males may be using other, less conspicuous cues that were not tested. For example, morphometric analysis may reveal other important morphological differences between the species that males use in species recognition. Although individual cues may not be sufficient for species recognition, this study is important to understanding the complex process of species recognition in a system where males risk mating with heterospecifics and suggests that male *P. latipinna* require a suite of cues for species recognition.

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